Winter 12-2011

Influence of Ionic Mobile Phase Additives with Low Charge Delocation on the Retention of Ionic Analytes in Reversed Phase HPLC

Cesar Florez
Seton Hall University

Influence of ionic mobile phase additives with low charge delocalization on the retention of ionic analytes in reversed phase HPLC

By:

Cesar Florez

Dissertation submitted to the Department of Chemistry and Biochemistry of Seton Hall University in partial fulfillment of the requirements for the degree of

DOCTOR of **PHILOSOPHY**

in

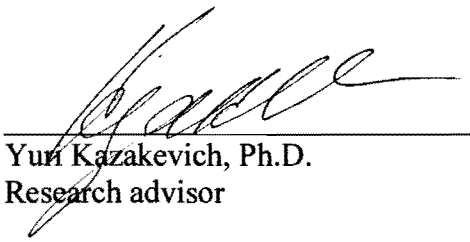
Chemistry

December 2011

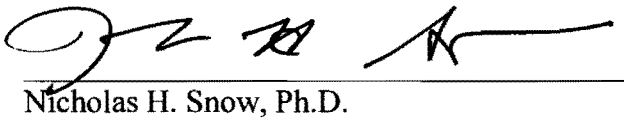
South Orange, New Jersey

We certify that we have read this thesis and that in our opinion it is sufficient in scientific scope and quality as a dissertation for degree of Doctor of Philosophy.

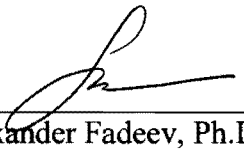
APPROVED:



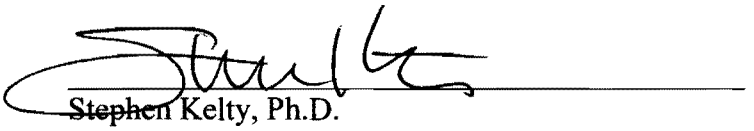
Yuri Kazakevich, Ph.D.
Research advisor



Nicholas H. Snow, Ph.D.
Member of Dissertation Committee



Alexander Fadeev, Ph.D.
Member of Dissertation Committee



Stephen Kelty, Ph.D.
Chair of Chemistry and Biochemistry department.

Acknowledgements

First and Foremost, I thank God for giving me the opportunity to expand my knowledge and engage in graduate studies and for putting the right people in the right place and at the right time - when I needed His guidance the most. Amen.

My sincerest gratitude is allotted to my advisor, Professor Yuri Kazakevich Ph.D. for the continuous support on my doctoral studies and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance was critical throughout the endeavor of researching and writing of this thesis – an accomplishment that could not have been possible without him. Thank you.

I would like to thank the rest of the thesis committee: Professor Nicholas Snow Ph.D. and Professor Alexander Fadeev Ph.D. for their encouragement, insightful comments, and hard questions.

My sincere thanks also goes to Yadan Chen and Lisa Wright for offering me invaluable support during my thesis writing, for offering research opportunities in their groups, their guidance, and allowing me to partake in projects at the Merck Research Institute. I warmly appreciate their generosity and understanding during my doctoral studies.

I thank my fellow lab mates of the analytical research group at Seton Hall University for the stimulating discussions and for all the fun we have had during my years in graduate school.

I would like to thank my parents, Marco Tulio and Maria Teresa and my sister, Isabel Christina for supporting me spiritually throughout my life, guiding me in every step and for providing me with the basic education that catapulted me to take the next step of the doctorate degree.

Last, but not least, my wife, Tara Rose and my children, Daniel Fernando, Sofia Rose and Natalia Maria who helped me stay sane through these difficult years. Their support and care helped me overcome setbacks and stay focused on my graduate studies. I greatly value their love and deeply appreciate their belief in me. None of this would have been possible without their love and patience. They have been a constant source of love, concern, support and strength all these years. God bless them.

Your time is limited, so don't waste it living someone else's life. Don't be trapped by dogma, which is living with results of other peoples thinking.
Don't let the noise of other's opinions drown out your own inner voice, and most important, have the courage to follow your heart and intuition, they somehow already know what you truly want to become, everything else is secondary.

Steve Jobs.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1-Introduction

1.1-Amphiphilic ions and ion pairing

One of the biggest challenges facing scientists working on chromatography is the separation and identification of mixtures of ionizable and ionic analytes. Since the protonation of these compounds is pH dependent, the use of reversed phase-high performance liquid chromatography (RP-HPLC) is restricted to the use of mobile phases in which the pH of the aqueous portion of the mobile phase is fixed depending on the pKa of the compound. A classic approach used to separate charged analytes is ionic suppression[1].

Ionic suppression technique is based on the pH adjustment of the mobile phase which results in a non-ionized analyte. However, this adjustment of pH is only suitable for single compounds or simple mixtures of bases where the pKa's of the compounds are close together[4].

Ion exchange chromatography is an alternative separation method for ionic analytes. This separation is performed on stationary phases containing ionized groups oppositely charged to the ionic analyte. In this technique the

selectivity is limited because the hydrophobic moiety of the charged species does not strongly contribute to the analyte retention driving force.

Ion-interaction chromatography is an intermediate between reversed phase and ion-exchange chromatography. In this technique, the eluent mixtures contain amphiphilic or liophilic ions that cause their adsorption on the hydrophobic surface of the stationary phase followed by the formation of a pseudo ion-exchange surface. Charged analytes can interact with the counterions in both the pseudo ion-exchange surface and the mobile phase. Ionic interactions are critical in regard to analyte retention, solvation, ionic equilibrium and other processes when utilizing reversed phase HPLC with water/organic mobile phase mixtures[1-3].

1.1.1-Amphiphilic compounds

Amphiphilic ions contain a charged group attached to an alkyl chain. These ions are also known as surfactants. In the chromatographic separation process, these ions are added to the mobile phase and they accumulate on the surface of the stationary phase. These ions are oriented at the interface between the hydrophobic surface of the stationary phase and the eluent, and the orientation of them have the charged end of the alkyl surface in the eluent and the hydrophobic alkyl chain adsorbed on the stationary phase. This orientation of the amphiphilic ions allows a formation of a charge surface.

The excess surface charge is compensated by the accumulation of counter ions in the mobile phase at the close proximity of the surface forming an electrical double layer. Retention of charged analytes using amphiphilic ions have been observed by many scientists for decades[1,2,3]

Several names have been used for this technique: Soap chromatography, solvent generated ion-exchanged, ion interaction and ion-pair chromatography. With the introduction of many names, a number of different theories were introduced to describe the effect of the introduction of amphiphilic ions into the mobile phase. Two main theories can be described: stoichiometric and non stoichiometric[4].

1. Stoichiometric models

All the stoichiometric retention models are based on the same structure of the adsorbed complex and direct to the same mathematical descriptions[5]. Three main stoichiometric models of retention should be mention: classical, dynamic and comprehensive.

1.a. Classical

In the classical model, an ion pair reagent combines with the analyte in the eluent and form a neutral species, this species then partitions onto the hydrophobic stationary phase[6,7]

1.b. Dynamic ion exchange

In the dynamic ion exchange, an ion pair reagent adsorb on the surface of the stationary phase, this adsorption of ion pair reagents produce charge sites which will create exchange places for analyte interaction.[8].

1.c. Comprehensive

Other models are based on combinations of the classical and the dynamic ion exchange mechanisms[9,10]. There is a probability that both mechanisms coexist and that the extent to which one is more dominant than the other one is not exactly known[9].

Based on the Bidlingmeyer model[11], an ion pair reagent is considered under isocratic conditions and it dynamically gets adsorbed on the hydrophobic surface of the stationary phase, this adsorption of ions creates a primary charged ion layer on the surface of the stationary phase, the counter ions then, are located in the diffuse outer layer forming an electrical double layer.

The retention of the analyte involves a mix mode mechanism of transfer of analyte through this electrical double layer and Van Der Waals forces.

2. Non-Stoichiometric Models

Two models can be considered in this retention model, Stahlberg[12,13] and Cantwell[14,15]. These theories only consider the process to be based entirely on formation of an electrical double layer and they ignore ion-pairing process in the bulk eluent.

2.a. Stahlberg electrostatic model

This description of the electrical double layer is based on Gouy-Chapman theory which describes the electrostatic potential profile as a function of distance from surface. In this model, two layers are under dynamic equilibrium, the primary layer is due to the adsorption of the ion pair reagent and the second layer is diffuse and contains the ion pair reagent counter ion. In this model, analyte retention is directed by the free energy of adsorption of the analyte, which is the addition of two contributions, the chemical part measured as the analyte hydrophobicity (free energy of adsorption of analyte in the absence of ion pair reagent) and the electrostatic contribution represented by the work involved in the transfer of charged analyte to the charged stationary phase.

2.b.Cantwell model

The Cantwell model is more complex than the Stahlberg model, this model is based on ion-exchange and interaction with the electrical double layer[16], Cantwell description of the electrical double layer is based on Stern-Gouy –Chapman theory and it takes into account that counter ions are not able to get closer to the surface than the plane of closest approach of counter ions. The main process of analyte retention is an ion exchange of between the bulk of the mobile phase and the diffuse part of the diffuse layer.

As the stoichiometric models disregard the demonstrated progress of the electrical double layer, non-stoichiometric models overlook the experimental proof of the formation of ion-pairs species in between analytes and ion pair reagents. In addition, hydrophobic ion-pairing equilibria cannot be explained by purely electrostatic models[17] .

The major drawback of these theories is that they are derived for the flat open adsorbent surface. However, chromatographic separations are based on porous material adsorbents. Chemical modification reduces the effective pore diameter of porous materials; the properties of the electric double layer for this confined space are different from the electric double layer for flat surfaces.

Two mechanisms of analyte retention could be envisioned: the first mechanism is the formation of ion pair between the analyte and amphiphilic counterion with subsequent adsorption of the formed complex on the surface of the stationary phase, and the second mechanism is the adsorption of the amphiphilic counterion on the stationary phase surface follow by retention of charged analyte in an ion-exchange mode. Melander and Horvath[18] concluded that mostly likely both mechanisms coexist in the chromatographic retention process.

1.1.2-Phenomenological treatment of ion pairing

1.1.2.1-Ion-pair definition

An ion pair can be illustrated as two ions of opposite charge which are temporarily held together by an electrostatic interaction without forming a chemical bond, in other words a pair of oppositely charged ions held together by Coulomb attraction without formation of a covalent bond.

An ion-pair is considered as such if the distance between two ions of opposite charge in solution is lower than a critical distance, ion–pair components cannot approach each other closer than the distance of the closest approach due to the strong repulsive interactions of their corresponding electron shells.

1.1.2.2-History and development of ion-pair theory

Arrhenius theory in the early 1880's set the beginnings of the ion-pair model. His theory describes the electrolytic dissociation in solution as a function of electrolyte concentration and character. Debye and Hückel[19] initiated the first fundamental approach in 1923 to determine the thermodynamic properties of strong electrolytes. This approach has become the basis for theoretical and practical applications[20,21]. Due to the intrinsic approximations in their theory and electrolyte model, the Debye and Hückel approach is restricted mainly to univalent salts at low concentrations.

Bjerrum in 1926, proposed a correction with an entirely different approach endorsing Brönsted's idea of specific ionic interactions[22] . This different approach included an ion-pair association which extended the validity of the Debye-Hückel result to solvents with a lower dielectric constant. Even though this correction has been criticized by theoreticians due to its empirical aspect it has been used by experimenters because of its indisputable success. The basis of his theory was the introduction of a chemical model (ion association) into the results obtained from a non-rigorous treatment (linearized Poisson-Boltzmann equation) of a Hamiltonian model (charged, non polarizable hard spheres in a continuum).

In Debye- Hückel theory, if a counter ion in the ionic cloud becomes close to the central ion during random thermal movement, its thermal translational energy won't be enough to continue the ion's independent movement in

solution, since the two ions will be trapped in each other's electric field. Ions of opposite charge stay together after collision for a short period of time, this concept is known as ion-pair

The association concept or ion pair assumes that a fraction of anion-cation couples lose their electrolytic properties when their Coulombic attraction is strong, in the same way as weak electrolytes do but for different reasons.

The ions are considered to be in chemical equilibrium and characterized by a thermodynamic association constant, K_A. This constant could be evaluated and define the association of anion and cation pair as this for which the separation distance r obeys the following relations:

$$0 < r < R$$

R = arbitrary distance, then,

The thermodynamic association constant can be expressed as a function of anion-cation pair potential, radius and temperature as follows:

$$K_A = \frac{N}{1000} 4\Pi \int_0^R r^2 e^{\left(-\frac{W_{(r)}}{kT}\right)} dr \qquad 1$$

Where,

$W_{(r)}$ = anion-cation pair potential

$\frac{N}{1000}$ = factor necessary to obtain K_A in moles/Liter, where N is Avogadro number

Bjerrum treated this model as a primitive model identifying the potential W with the isolated anion-cation pair potential $u_{(r)}$

$W_{(r)} = u_{(r)} = \infty$ when $r < a$

$$W_{(r)} = u_{(r)} = \frac{Z_1 Z_2 e^2}{rD} \text{ when } r \geq a \qquad 2$$

For the upper limit of the integral, Bjerrum chose the distance where the integral goes through a minimum and the final results looks like:

$$R = -\frac{Z_1 Z_2 e^2}{2DkT} = q \qquad 3$$

This will give the final result for the thermodynamic association constant.

$$K_A = \frac{N}{1000} 4\Pi \int_a^q r^2 e^{(\frac{2q}{r})} dr \qquad 4$$

q represents the distance for which the probability of finding a counter ion in a spherical shell next to the central oppositely changed ion is minimum. The probability increases for lower distances even if the number of ions in the shell thickness is very low, in this case the Coulombic attraction will be stronger.

In the case of higher distances, the probability increases due to the volume of the spherical shell and the greater number of ions in the spherical shell; but in this case, due to the lower electrostatic force ion-pairs are not formed since the ions are unable to stick together because they are scattered apart by thermal motion.

1.1.2.3-Classification of ion-pairs

Ion pairs in solution can be classified depending on the interaction between cation, anion and the surrounding solvent molecules in three different categories: Solvent separated ion-pair, solvent shared ion-pair and contact ion-pair.

1-Solvent separated

An ion pair whose constituent ions are separated by one or several solvent or other neutral molecules is described as a solvent separated ion pair, this ion-pair can be represented as $A^+||B^-$. The components of a solvent separated ion pair can readily interchange with other free or loosely paired ions in solution. Isotopic labeling can be used to experimentally distinguish between tight and loose ion pairs.

In the solvent separated ion-pair, each ion maintains its own primary solvation shell.

2-Solvent shared

There is an additional distinction between types of loose ion pairs. When the ionic constituents of the Ion-Pair are separated by only a single solvent molecule it is known as solvent-shared ion pairs, while in solvent-separated ion pairs more than one solvent molecule are involved.

3-Contact ion-pair

When the cation and the anion are in direct contact and not separated by an intervening solvent or other neutral molecule is designated as a tight ion

pair (intimate or contact ion pair). A tight ion pair of A^+ and B^- could be symbolically represented as A^+B^-.

1.1.2.4-Experimental determinations of ion pairs

The evidence of ion-pairs has been reported by many different analytical techniques with life times as low as 1 ns[23,24,25,26,27]. The first evidence of ion-pair was obtained by conductometry measurements[24], this technique has been established as a reliable source for the determination of the presence of ion-pairs, Fuoss and Hsia equation [25] is one of the most popular ones, even though this one has been extended by Fernandez P and Justice[26] . It has been accepted that electro neutral ion-pairs do not contribute to solution conductivity. The main disadvantage of conductometric measurements is that as the ion-pair association constant decreases so is the reliability of the measurements. Another technique that has been used for the determination of ion-pairs is capillary electrophoresis [27,28,29], ion association constants can be determined by measuring the retention time shift of the analyte peak; the capillary electrophoretic mobility of large anions decrease with increasing concentration of large lipophilic cations in the background electrolyte.

The presence of ion-pairs has been observed in some other experimental techniques such as: Potentiometry[30], Ultrasonic Relaxation[31], Dielectric Relaxation Spectrometry[32], UV-Vis[33], IR[34], Raman[34] and NMR[34].

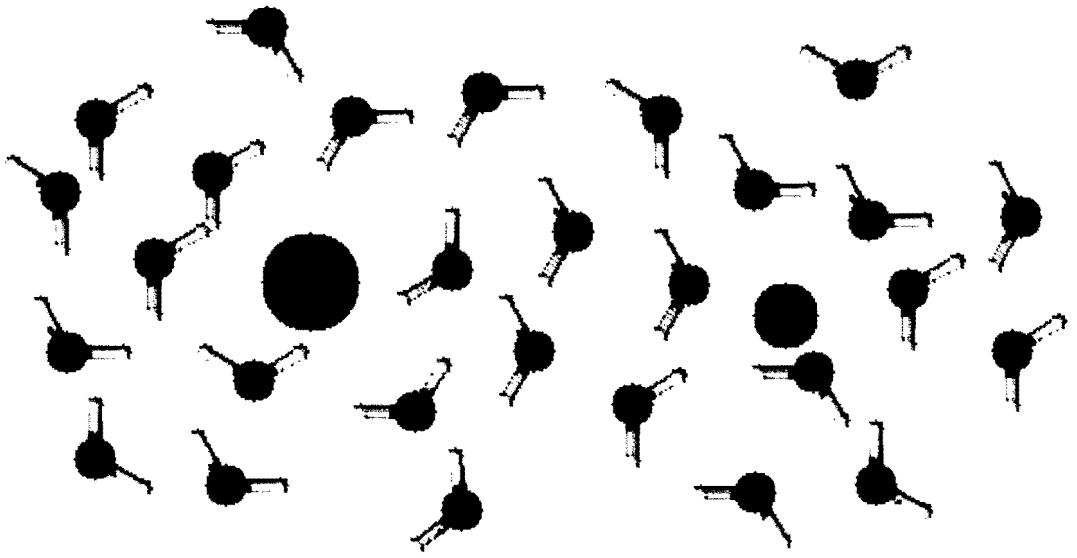


Figure 1. Representation of a solvent separated Ion-Pair. Anion (green) and cation (grey) are separated by multiple layers of solvent. Figure was drawn using ChemSketch from ACDLABS version 11.0.

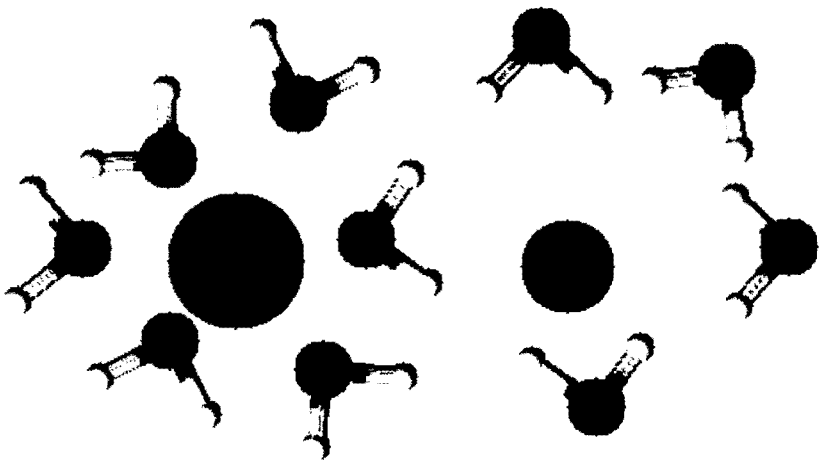


Figure 2. Representation of a solvent shared Ion-Pair. Anion (green) and cation (grey) are separated by a single solvent layer. Figure was drawn using ChemSketch from ACDLABS version 11.0.

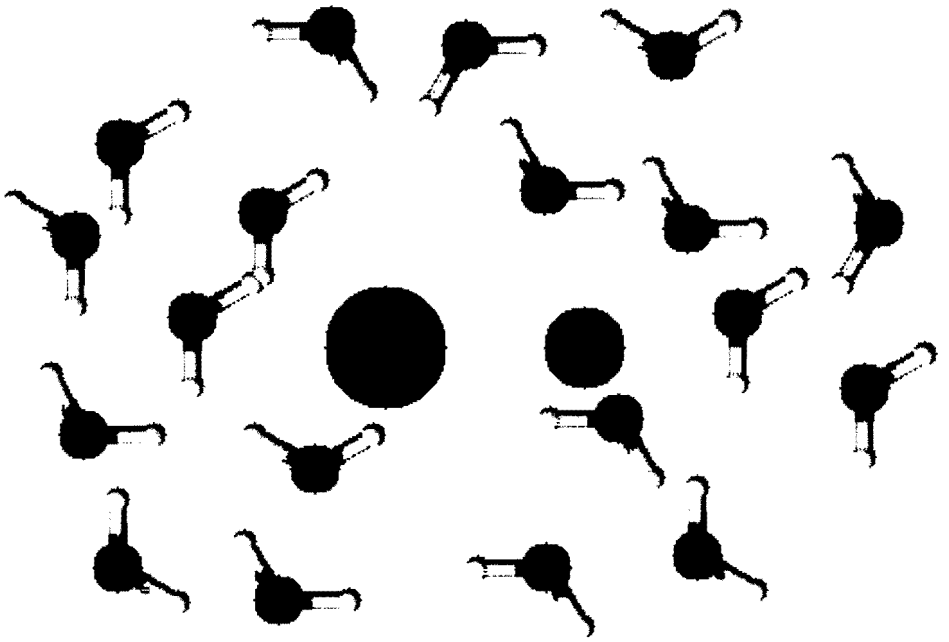


Figure 3. Representation of a contact Ion-Pair. Anion (green) and cation (grey) with no solvent molecules in between ions. Figure was drawn using ChemSketch from ACDLABS version 11.0.

1.2-Chaotropic theory

Although the use of amphiphilic reagents in ion interaction chromatography provides an excellent alternative for the retention and separation of ionic and ionizable analytes, it is often suggested as the last resource for the retention of ionic and ionizable analytes. Addition of this type of reagent to the mobile phase for the retention of very hydrophilic compounds produces an irreversible adsorption of this reagent on the surface of the reverse phase adsorbent, the amount of pairing agent adsorbed by the stationary phase from the eluent has been determine using a method described by Knox and Hartwick[35].

Similar effects on retention of protonated analytes have been achieved with small inorganic ions which are characterized by significant charge delocalization, symmetry, and overall electron density. These ions known as liophilic ions show relative weak interactions with alkyl chains on the bonded phase and have significant dispersive interactions.

The presence of these small inorganic ions known as liophilic ions in aqueous solution was found to disrupt the water structure[36].These ions create a disruption of the solvation shell and therefore create chaos (an schematic representation of chaotropic effect can be seen in Figure 4) , these small inorganic ions, which create chaos, are known as chaotropic ions. The addition of these ions into aqueous solutions was found to increase chaotropicity or chaos into the structured ionic solution[37,38].These ions are

usually spherical in shape and characterized by significant charge delocalization.

1.2.1-Chaotropic representation

Chaotropic agents used in reversed-phase HPLC are usually small inorganic ions such as BF_4^-, CF_3COO^-, ClO_4-, and $H_2PO_4^-$, with liophilic nature. Liophilic ions are described in literature[39] as having significant delocalization of the charge, symmetry, spherical shape, and absence of surfactant properties. The effect of the addition of these chaotropic ions in aqueous solution was explained as disruption of the water structure or introduction of chaos into structured ionic solution[39],[40]. This effect was firstly observed by Franz Hofmeister[41].Different ions have different ability to disrupt solvation shell and they are arranged according to the Hofmeister series as follows,[42] $H_2PO_4^-$< $HCOO^-$ < $CH_3SO_3^-$ < Cl^- < NO_3^- < CF_3COO^- < BF_4^- < ClO_4^- < PF_6^-. From the left to the right, the chaotropicity increases with an increase in hydrophobicity, charge delocalization, symmetry, and overall electron density.

The solvation of the protonated basic analytes decreases with the increase of the counterion concentration in the mobile phase. The decrease of the analyte solvation by disrupting the primary sheath of water molecules around the analyte increases the analyte hydrophobicity; an increase in the hydrophobicity of the analyte augments the interaction with the hydrophobic surface of the stationary phase. This increase in hydrophobicity leads to enhance in retention of the basic protonated analytes.

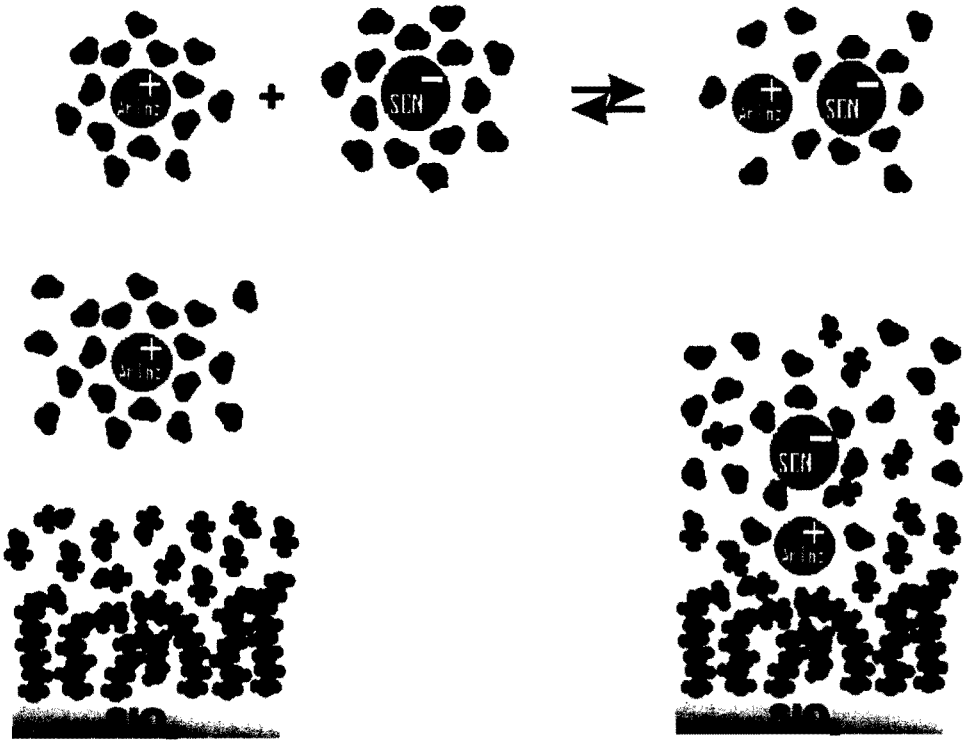


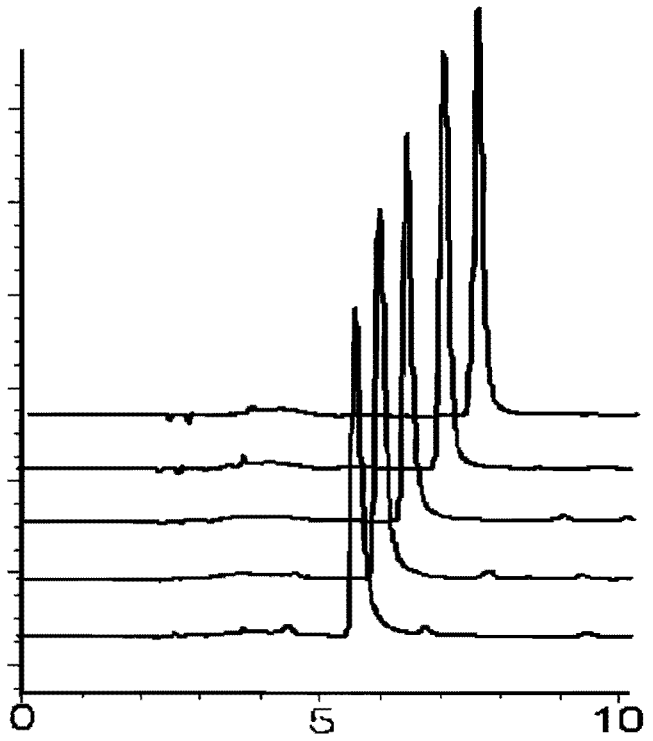
Figure 4. Representation of the chaotropic effect. Retention mechanism of protonated aniline on reversed-phase material in water/acetonitrile eluent in the presence of chaotropic ion thiocyanate. This mechanism shows the disruption of the solvation shell of completely solvated aniline (top and bottom left) and the creation of ion-associated specie (top and bottom right) with higher hydrophobicity.

The pH of the solution is an important factor in the development of a chromatographic method, since pH controls the ionization degree of the analytes and therefore the magnitude of electrostatic interactions that can be use to regulate retention.

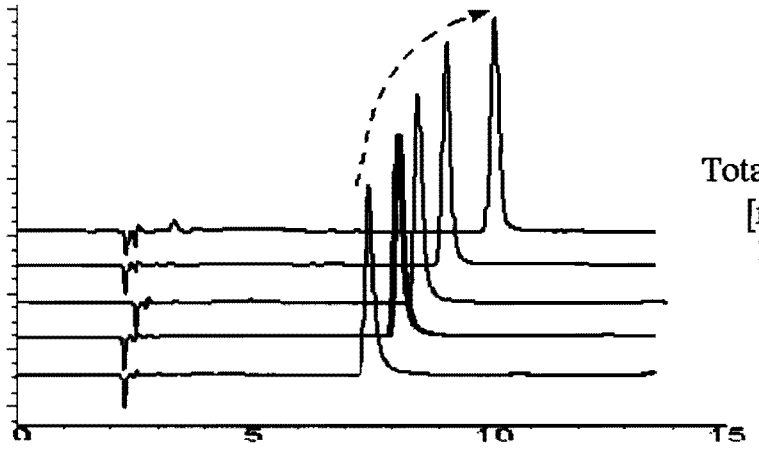
It has been observed[36] that at low pH where basic analytes are completely protonated, any additional decrease in pH due to the addition of acid produced a similar retention pattern as just the addition of chaotropic salt at a constant pH. It was concluded the chaotropic effect observed was due to the concentration of the chaotropic acidic modifier counter ion and not to the pH change, and that this change will depend on the nature of the anionic species and not the sources of the anion like salt or acid

It has been noticed that the increase of the retention of the protonated basic analyte is dependent on the concentration of the chaotropic counter anion and not on the concentration of protons in solution as long as the pH of the analyte is below its pKa as it can be seen in Figure 5.

Increase of analyte hydrophobicity leads to an increase in analyte retention. This process shows a saturation limit when the counteranion concentration is high enough to effectively disrupt the solvation of all analyte molecules. Additional increase in counteranion concentration does not produce any noticeable analyte retention.



Total ClO_4^- [mM]	pH
47	1.4
41	1.5
20	1.8
14	2.0
7	2.2



Total ClO_4^- [mM]	pH	Rt. (min.)
100	2.0	9.9
89	2.0	
79	2.0	
70	2.0	
55	2.0	7.7

Figure 5. Effect of retention of aniline when perchloric acid is used as the acidic modifier throughout pH 1.3-7.1. Column 150x4.6 mm Zorbax XDB-C_{18} mobile phase: acetonitrile-10mM disodium hydrogen phosphate buffer adjusted with perchloric acid, pH 1.3-7.1 (10:90): flow-rate, 1.0ml/min; 25°C;UV, 254nm; sample: 1µl injection. (Reprinted from reference 36 with permission)

The analyte solvation-desolvation equilibrium could be expressed in the following form:

$$B_s^+ + A^- \Leftrightarrow (B^+.....A^-) \qquad \textbf{5}$$

B_s^+ = solvated basic analyte

A^- = counteranion

$(B^+.....A^-)$ =desolvated ion-associated complex

If solvation-desolvation process is assumed to be in a fast equilibrium, the overall retention factor for analyte can be expressed as a function of the desolvation parameter, retention factor for solvated and desolvated analyte and the concentration of counteranion[36] as follows:

$$k = \frac{k_s - k_{us}}{K[A^-]+1} + k_{us}, \qquad \textbf{6}$$

k_s = limiting retention factor for solvated analyte

k_{us} =limiting retention factor for desolvated analyte

K =desolvation parameter

$[A^-]$ =Counteranion concentration

Experimental dependency of retention for basic protonated analytes as a function of counteranion concentration has a good agreement with the theoretical value calculated using Equation 6 and the typical retention behavior can be seen in Figure 6.

Temperature is an important factor for ion interaction chromatography since adsorption isotherm depends on it. J. Li wrote a great theoretical analysis evaluating the effect of temperature on selectivity for various retention mechanisms[43]. Temperature is one factor that can affect the amount of adsorbed counteranion on the surface of the stationary phase, retention time of analyte is affected by the amount of adsorbed counteranion on the surface. Some of the variables affected with change in temperature are: ionization of solutes, ionization of buffer components and value of thermodynamic equilibrium constant From the thermodynamic point of view, chaotropic and kosmotropic ions can be classified depending on the change in the mobility or entropy of water molecules caused by the presence of ions in solution[44]; the negative entropy change caused by the addition of ions can be plotted as a function of radius of the ions, see Figure 7. The point where the entropy change is zero can be defined as the ions with the intermediate strength or intermediate chaotrope/kosmotrope characteristic, this horizontal line, separates small ions that bind water strongly $\Delta S > 0$ from large monovalent ions that bind water weakly $\Delta S < 0$. The radius for cations (1.06 Å) and anions (1.78 Å) represents ions that do not change the entropy of nearby water

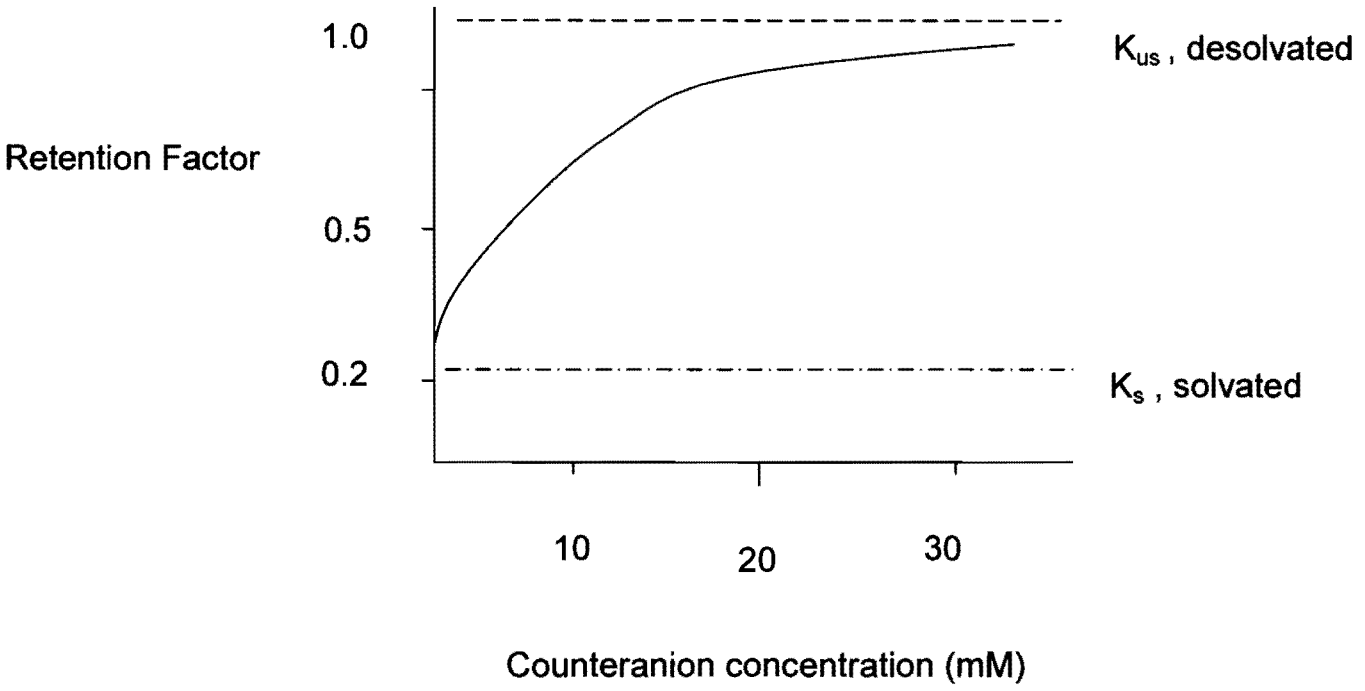


Figure 6 variation of retention of basic protonated analyte as a function of counteranion concentration.(reprinted from reference 36 with permission).

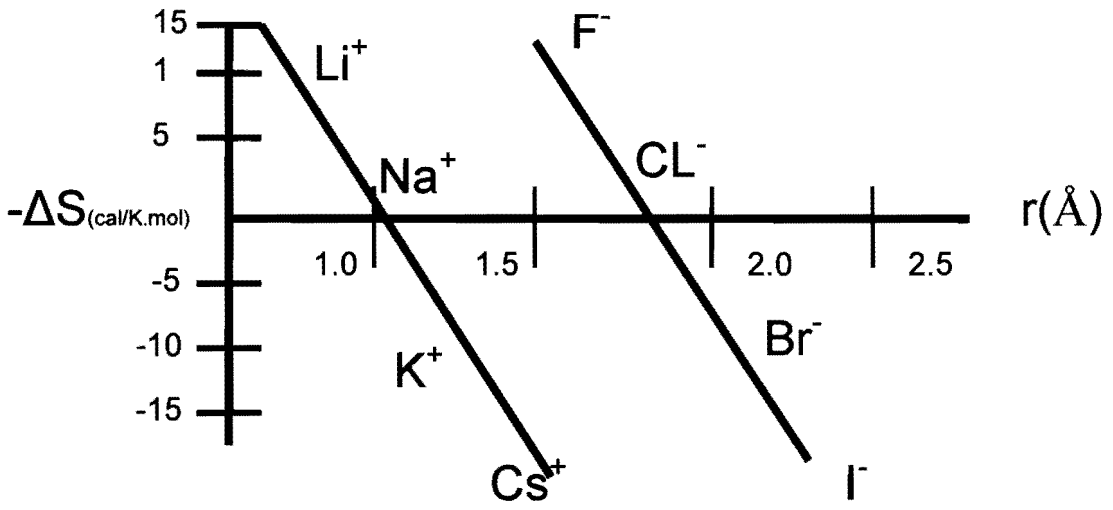


Figure 7. Difference in the entropy of water near an ion minus entropy of pure water ($cal*K^{-1}*mol^{-1}$) plotted versus radii of ions (Angstroms) for different ions from the Hofmeister series (reprinted from reference 44 with permission).

molecules; the ions above $\Delta S = 0$ decrease the mobility of nearby water molecules, these ions are called kosmotropes. On the other hand, the ions below this horizontal line increase the mobility of nearby water molecules, these ions are called chaotropes. Anions are more strongly hydrated than cations for a given charge density, since the anions begin to bind the immediately adjacent water molecules strongly at a lower charge density.

There are at least two reasons for the stronger hydration of the anions. First, quantum mechanical calculations indicate that the anions, which interact with the hydrogen atom of water, allow intra shell hydrogen bonding of the solvating waters, whereas cations, which interact with the oxygen atom of water, do not. Second, charge transfer to solvent characterizes strong hydration; because the oxygen atom of water is very electronegative, it is easier to accept negative charge from anions than positive charge from cations[44].

Small ions are strongly hydrated because their point charge is close to the point charge of opposite sign on the water molecule, whereas large ions are weakly hydrated because their point charge is distant from the point charge of opposite sign on the water molecule It is energetically unfavorable to strip a water molecule off of a small ion, but the energy lost is more than regained when another small ion takes the place of the water molecule, because the two point charges of opposite sign in the newly formed neutral salt are closer together. Therefore, formation of small-small inner sphere ion-associated complex is energetically favorable. Similarly, although the interaction

between the distant point charges of a large-large ion-associated complex is weak, removal of water molecules from large ions leads to new water-water interactions that are stronger than large ion-water interactions, and thus formation of large-large inner sphere ion-associated complex is also energetically favorable. In contrast, the work done in stripping a water molecule off of a small ion is not regained by replacement with a large ion, because the point charge of the large ion is too distant from the point charge of the small ion to interact strongly with it. Consequently large-small ion-associated complex tend not to form inner sphere ion-associated complex; they remain apart in aqueous solution and as a result are highly soluble. An aqueous solution consisting of ions of various sizes will tend to segregate according to size. The small ions of opposite sign and comparable size will tend to pair because they form stronger interactions than those between large ion-associated complexes; the medium-sized ions of opposite sign and comparable size will also tend to pair because they form stronger interactions than those between medium-large ion-associated complex; and the large ions of opposite sign and comparable size will also tend to pair because their formation releases water for formation of stronger water-water interactions. Protonated basic analytes will form different ion pair species depending on the characteristic of the analyte and the characteristic of the chaotropic/kosmotropic anion added to the mobile phase. It is of our interest to evaluate the retention of the different ion-associated complexes formed and

to correlate the chromatographic retention of these ion-associated complexes with the position of the chaotropic/kosmotropic ion in the Hofmeister series.

1.2.2-Effect of chaotropic ions on analyte retention

The influence of the chaotropic ions on the retention of basic protonated analytes can be described with three possible different mechanisms[45]:

1. Ion pairing which involves the formation of neutral ion pairs and their retention based on the hydrophobic reversed-phase mechanism.

2. Chaotropic model, in which counteranions disrupt the analyte solvation shell and lead to an increase in its apparent hydrophobicity and retention.

3. Liophilic counteranions are adsorbed on the surface of the stationary phase, thus these introduce an electrostatic component into the general hydrophobic analyte retention mechanism.

Guiochon and co-workers support the domination of classic ion pairing process[46],[47],[48]. They explain the effect of the counterion on the basis of the formation of a neutral ionic complex, which in then adsorbed on the hydrophobic surface of the stationary phase.

Horvath[49],and Sokolovski[50],[51] developed a comprehensive theory for the retention of ionic compounds based on stoichiometric adsorption of ionic species on reversed phase columns, Stählberg[52] on the other hand based his

theory more on the form of adsorption of ions and formation of an electrical double layer, these theories are essentially regarded as ion-pair chromatography,

All three mechanisms mentioned above probably exist but one of them might be the dominating and this will depend upon the eluent type, composition, and adsorbent surface properties.

Inorganic ions were arranged in a series according to their ability to disrupt the water solvation shell; this series is known as Hofmeister series [7]. Originally the degree of the effect of chaotropic ions on the analyte retention was associated with its position on Hoffmeister series[70]. Hoffmeister series contains two distinct types of ions: chaotropes and Kosmotropes, Chaotropes being disruptive for water structurisation and Kosmotropes being facilitative for water structurisation.

1.2.3-Hofmeister series for chaotropic and kosmotropic ions

Hofmeister noted that cations and anions can be ordered based on their effect on the solubility of proteins[53].The so-called Hofmeister series ordering has been further extended to include the effect of ions on a wide range of application[54] including chromatographic separations. Since its initial description there have been considerable interests and activities directed at exposing the underlying mechanism behind the Hofmeister series ordering of ion specific effects; nevertheless, the origin of this ordering remains uncertain and controversial[55,56]

One of the proposed mechanisms for the Hofmeister ordering is through ion specific alterations in the hydrogen bonding network of water[57]According to whether the ions are strongly hydrated or weakly hydrated, ions have been classified as either kosmotropes (structure makers) or chaotropes (structure breakers) respectively[58,59] Though supported by indirect thermodynamic and macroscopic experiments[60,61,62,63,64,65,66,67,68] the prevailing mechanism based on ion induced alterations in water interactions have been re-evaluated and challenged in recent studies that indicate a lack of direct effect of ions on bulk water[69,70,71,72,73,74]. Molecular simulation studies have provided contradictory descriptions based on chosen models and initial parameters[75,76].

A possible reconciliation among these two different views and sets of results relating to the origin of the Hofmeister series might emerge from recent findings that ions accumulate and perturb local water structure at

surface interfaces. The degree of ion accumulation appears to scale with their ordering within the Hofmeister series[77]. It is becoming ever more apparent that protonated analyte retention is somehow tied to hydration shell properties[78],[79],[80],[81],[82],[83]. Thus, potentially, ions that alter the hydration layer surrounding protonated solutes can impact their properties without significant perturbation on water interactions in the bulk solvent regime.

2-Problem statement

2.1-Kosmotropic effect on analyte retention:

Considering chaotropic influence as it is described by Flieger and Roberts [84] where analyte retention is directly related to the position of the counterion in the Hofmeister series, if the addition of chaotropic salts increase the retention of analytes due to the disruption of the solvation shell, the effect of the addition of a kosmotropic salt to the mobile phase should in theory should lead to the decrease of the analyte retention, this decrease may be due to the facilitation or molecular structurization of water solvation shell, making the analyte more hydrophilic.

This expected effect of decrease of analyte retention will be evaluated in the current work and also expanded to methanol/water eluent system.

2.2-Organic modifier effect on the retention of protonated basic analyte.

The addition of chaotropic ions in HPLC mobile phase was shown to significantly increase the retention of basic analytes in ionic form[25]. This process was rigorously studied and it was shown that not only the disruption of solvation shell affect the analyte retention but also the concentration of acetonitrile in the mobile phase[85,86]. Apparently the interaction of these chaotropic ions with acetonitrile molecules (solubility of chaotropic salts in

acetonitrile fences them to be retained in adsorbed acetonitrile layer, thus increasing the retention of analyte, which acts as counterion for chaotropic ions.

In some previous publications[31] it was shown that chaotropic effect does not work in MeOH/water systems. There are two significant differences while using methanol instead of acetonitrile on HPLC: methanol is protogenic and can participate in ions salvation; and methanol does not form multimolecular adsorbed layer.

Whether the retention happens in the form of ion-pair or the chaotropic ion embedded into the adsorbed acetonitrile layer which creates electrostatic attraction for its counterions remains an open question.

Our present work will attempt to demonstrate that the effect of increase in retention of protonated basic analytes with the addition of liophilic ions in the mobile phase can be obtained in acetontrile/water as well as methanol/water mobile phases.

2.3-Hofmeister series

The recent studies that have lead to a revision in thinking regarding the origin of the Hofmeister ordering have all focused on anions that have a chaotropic characteristic and their effect on chromatographic retention of protonated species. In contrast, the present study focuses on a more extended series containing both chaotropic and Kosmotropic salts and in an evaluation of a possible order from kosmotropic to chaotropic salts according to the salt effect on analyte's retention.

3-Experimental

3.1-Chemicals

All solutions were prepared using Milli-Q water (MilliPore) and Acetonitrile from Fisher Scientific (Fair Lawn, NJ, USA), optima grade. The following salts were used to prepared the analytical mobile phases: ammonium thiocyanate, ammonium formate, ammonium chloride, ammonium acetate, potassium phosphate monobasic, potassium sulfate, sodium chloride. Two ionazable analytes aniline and pyridine, and one cationic analyte PTMA-Cl (phenyltrimetyl ammonium chloride) were used in the chromatographic studies. All salts were obtained from Fisher Scientific (Fair Lawn, NJ, USA), certified grade.

3.2-Probe Analytes

The selection of the analytes was based on the requirement to demonstrate the retention of ionic and ionizable analytes at different organic concentrations in the mobile phase and two different pHs. Structures and pKs of the respective analytes tested are shown in Figure 8.

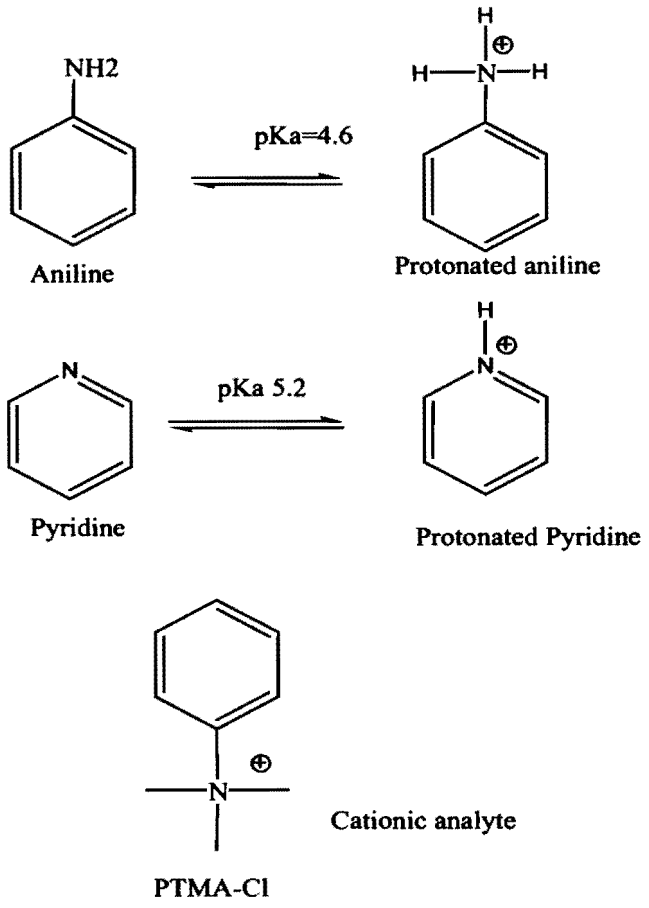


Figure 8. Aniline,pyridine and phenyltrimethylammonium chloride salt as model analytes.

3.3-Chromatographic conditions

The chromatographic system used to measure the retention of respective analytes was the separation module Alliance 2695 from Waters Corporation, (Milford MA); this system was equipped with an ultraviolet detector with a dual wavelength.

All the runs were performed using an analytical column from YMC-Pack Pro C18 (Kyoto, Japan), AS-300-3,S-3μm, 12nm, 50X4.6mm ID, S/N 040511295. The void volume calculated using deuterated acetonitrile and deuterated methanol method for this column was 0.58 mL. The chromatographic runs were performed under isocratic conditions at 25°C for the column temperature and 25°C for the sample temperature. The flow rate used was 1 mL/min. UV detection with a wavelength of 254 nm was selected for all experiments.

The organic modifiers selected for the chromatographic runs were methanol and Acetonitrile.

Analytes aniline, pyridine and PTMA-Cl were prepared at a concentration of 5 ppm in water. Injections of 10 μL of each of these three analytes were made.

Aqueous portion of the mobile phase was prepared with salt concentration ranging in between 1 mM and 100 mM.

The column void volume was measured according to the procedure described in[87] the comparison of the elution of deuterated acetonitrile from acetonitrile and deuterated methanol from methanol both show comparable values with less than 0.2% defference. The extracolumn volumes were accounted in all experiments.

4-Scope of the research

HPLC retention of ionic and ionizable analytes is known to be dependent on pH of the mobile phase, type of buffer added to the mobile phase and ionic strength. The influence of the type of salt and its concentration on the analyte retention has been attributed to a so-called "chaotropic" mechanism.

Out of the factors affecting analyte retention mentioned previously, we comment on the conventional understanding of parameters such as the effect of chaotropic ions and methanol as organic modifier as well as the strength of commonly used chaotropic ions.

Our recent studies have demonstrated that different inorganic ions don't influence analyte retention in the similar sequence as the position of these ions in Hofmeister series. In acetonitrile/water as well as methanol/water mobile phases all ions exhibit "chaotropic" effect, even the ions known to have "kosmotropic" properties (favorable for the formation of solvation shell) show the increase of the analyte retention with the increase of their concentration.

The present research is defined according to the following parameters:

4.1-Analytes used in the study

Two types of analytes were selected for the study, weak basic analytes (aniline and Pyridine) whose protonation is pH dependent and strong basic analyte (PTMA-CL) which remains protonated in the entire pH range.

4.2-Stationary phase used in the study

A conventional reversed phase C18 column (YMC-Pack Pro C18) was selected for the study to minimized ionic interaction between the analytes and ion pair reagents.

4.3-pH

Study of pH for weak basic analytes, aniline and Pyridine at pH 3.0, which is bellow pKa of analytes.

Study of pH for strong basic analyte, PTMA-CL, which remains protonated for the entire pH range.

4.4-Organic solvent

Effect of Acetonitrile/water (5:95) system was investigated for all analytes.

Effect of acetonitrile concentration from 5 % to 30% was studied for PTMA-CL. Effect of acetonitrile concentration was also studied as a

function of mobile phase temperature

Comparative studies were done for methanol/water and acetonitrile/water solvents for the retention mechanism of PTMA-CL which remains protonated for the entire operation range.

4.5-Ion-associated complexes

Total of nine chaotropic and kosmotropic reagents were evaluated for the effect on retention for aniline, pyridine and PTMA-CL

4.6-Chaotropic salt concentration

Effects of concentration for all nine chaotropic and kosmotropic reagents were evaluated ranging from 1mM to 100 mM

4.7-Temperature

Effect of column temperature on the retention of PTMA-CL was study ranging for 5 °C to 50°C.

5-Results and discussion

5.1-Effect of organic modifier and ammonium chloride concentration on the retention of protonated analyte PTMA-Cl.

Retention dependencies of Phenyl-trimethyl-ammonium chloride (PTMA-Cl) on the concentration of added salt at different acetonitrile/water mobile phases on a reversed phase column is shown in Figure 9. This quaternary amine was used as the main model analyte for this study since it's charge state is not affected by the mobile phase pH. The retention of $PTMA^+$ analyte cation, increases as the concentration of ammonium chloride (NH_4Cl) in the mobile phase increases. This trend is more pronounced at lower concentrations of acetonitrile in the mobile phase. A significant increase in retention is observed when the concentration of ammonium chloride increases from 0 to 5 mM.

Small ionic exclusion effect was observed when PTMA+ cation was eluted at high acetonitrile content and low ionic strength of the mobile phase (low concentration of ammonium chloride).

At a concentration of 10% of organic modifier in the mobile phase, acetonitrile forms an adsorbed layer of about 5 angstroms thickness as seen in

Figure 10 [88]and highly solvated analytes will definitely get excluded from this layer.

The thick adsorbed layer of acetonitrile provides a suitable media for the adsorption of liophilic ions on the stationary phase, this addition of liophilic ions creates an electrostatic component on the retention mechanism. On the other hand, methanol with the monomolecular layer should not significantly affect adsorption of ions.

As is can be seen in Figure 9, at all mobile phase conditions with acetonitrile/water, the addition of liophilic salt NH_4Cl exhibits increase in retention. At acetonitrile concentrations up to 30 v/v % $PTMA^+$ shows enhancement in retention, however, it was observed that at higher organic concentrations in the mobile phase, the retention of analyte starts to decrease, this behavior is attributed to the normal effect of the increase of organic composition in the mobile phase and the general dependence of the analyte retention on the eluent composition in the reversed phase HPLC, this decrease of analyte retention shows an exponential decay with the increase of the organic modifier concentration.

Phenyl-TrimethylAmmonium chloride retention as a function of liophilic ion concentration on the mobile phase at different acetonitrile concentrations.

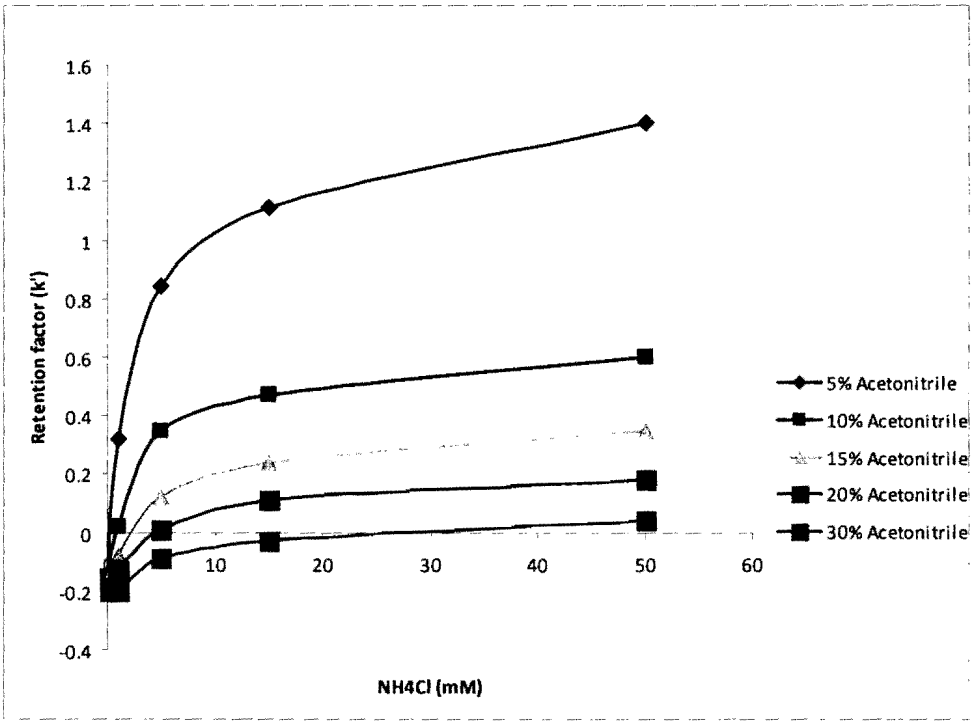


Figure 9. PTMA-Cl retention was evaluated as function of NH_4Cl concentration for different acetonitrile concentrations on a YMC-Pack Pro C18, AS-300-3,S-3µm, 12nm, 50X4.6mm ID, S/N 040511295 column,experiment run at room temperature, ammonium chloride added to the aqueous portion of the mobile phase.

Comparison of thickness of the adsorbed organic layer for methanol and acetonitrile.

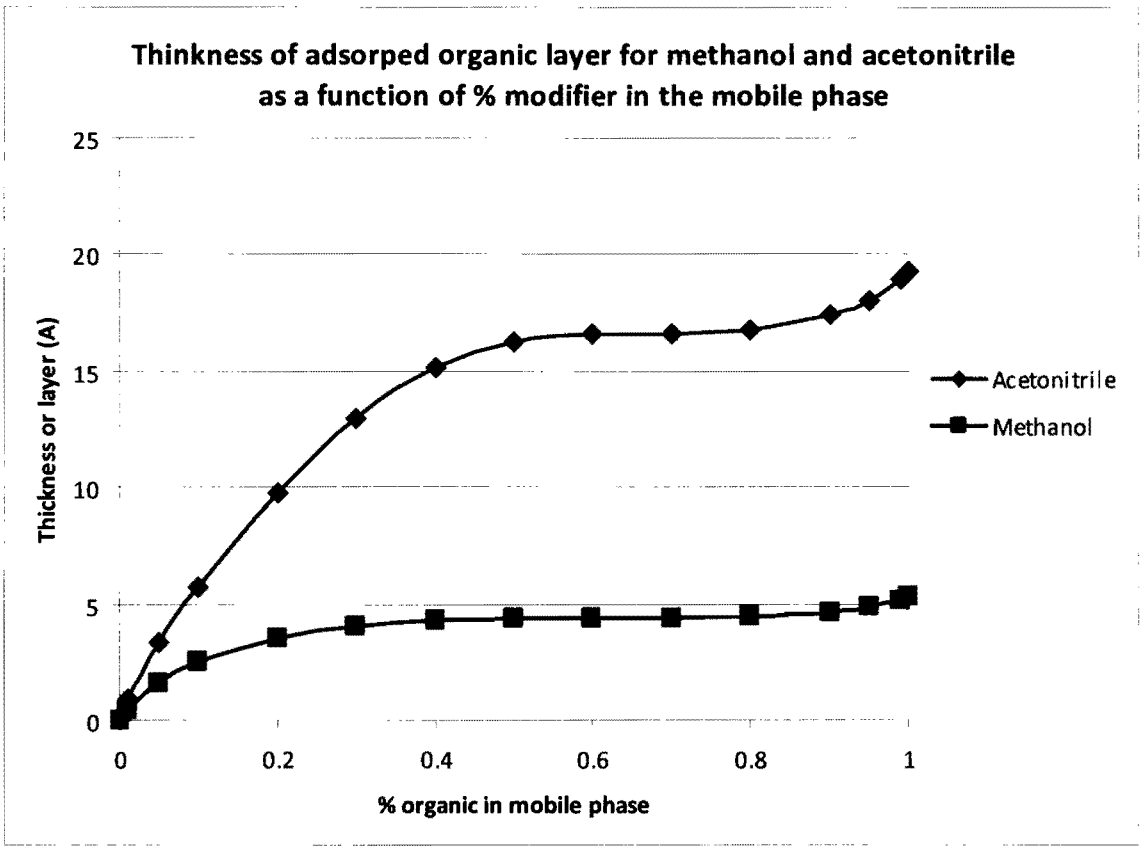


Figure 10. Experimental points for adsorption isotherms of methanol and acetonitrile.

Despite the very low effect of the chlorine concentration variation excerpt on the analyte retention this effect is still chaotropic, as the retention increases with the increase of the counterion concentration on the mobile phase.

This chaotropic trend was verified on its fit to the equation describing chaotropic retention effect derived by Kazavevich [36]

$$k(c) := k_1 + \frac{k_2 - k_1}{K \cdot c + 1} \qquad 7$$

Where k_1 is the limiting retention factor for completely solvated analyte, k_2 is that of desolvated analyte and K is the solvation constant. Limiting retention factors are shown in Table 1.

As it could be seen from Figure 11, Optimized theoretical curves correspond to the experimental points fairly well, which demonstrate that chlorine shows significant chaotropic effect.

Table 1. Limiting retention factors and Solvation constants optimized with MathCad GenFit function for application of equation 7 to the experimental retention data.

	$CH_3COO(NH_4)$	$HCOO(NH_4)$	NH_4SCN	K_2SO_4	KH_2PO_4	$NaNO_3$	NH_4Cl
k_1	1.248	1.668	4.769	0.756	0.689	1.674	1.519
k_2	0.443	0.409	0.489	0.35	0.381	0.371	0.372
K	0.198	0.121	0.172	0.251	1.176	0.065	0.055

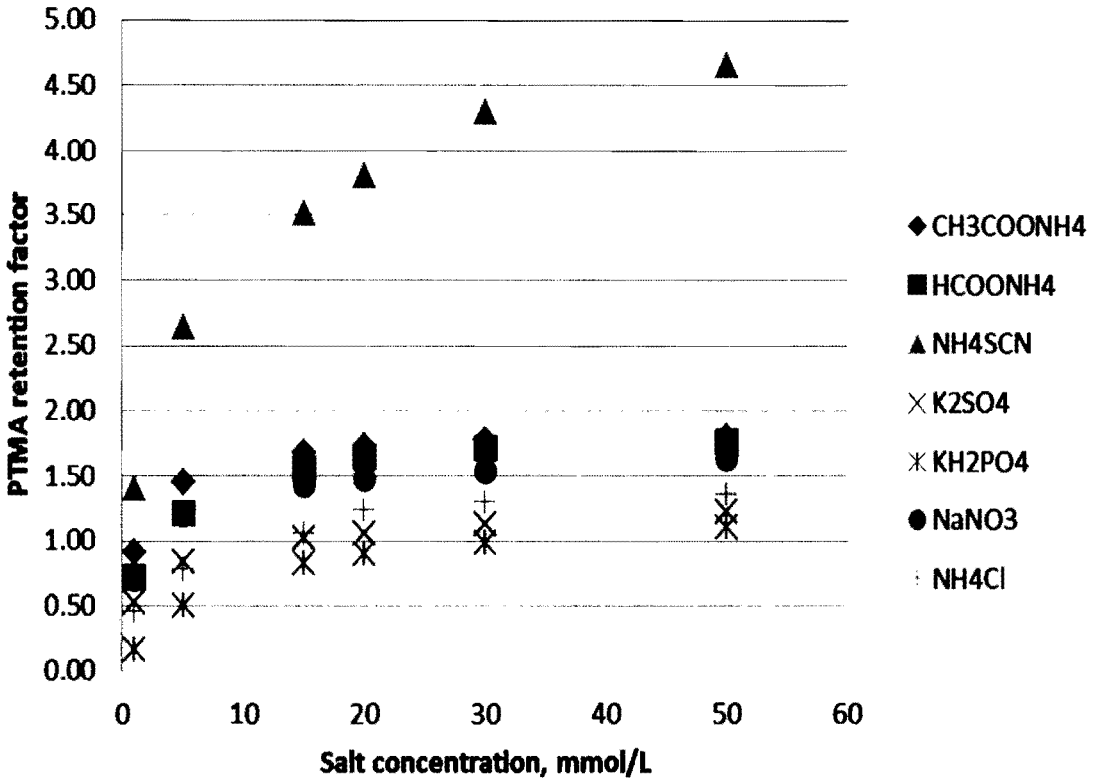


Figure 11. Variation of PTMA+ retention factor as a function of varios counterion concentrations. Column and conditions are the same as in Figure 12. Experimental data shown in this figure were optimized using GenFit function of MathCad for their suitability to equation 7.

5.2-Evaluation of protonated basic analyte retention with different liophilic salts

The retention of phenyl trimethyl ammonium chloride was also measured as a function of different liophilic ions concentration for all selected ions. Since low organic modifier in the mobile phase showed the highest effect on analyte retention with the addition of liophilic ion, this condition was selected as the most suitable to evaluate the effect of the addition of liophilic ions on basic analyte retention. Figure 12 demonstrates these experimental points.

It is interesting to note that the lowest effect on the analyte retention is from dihydrogen phosphate counterion but at the same time $H_2PO_4^-$ demonstrate strongest desolvation effect (highest desolvation constant). Also other ions demonstrate such influence on the analyte retention that does not correlate with their position on Hofmeister series.

Sulfate in its influence is very similar to phosphate except that the slope of the curve is much lover which is demonstrated by its low desolvation constant. Formiate in its position should be similar to phosphate while it demonstrates chaotropic properties close to acetate.

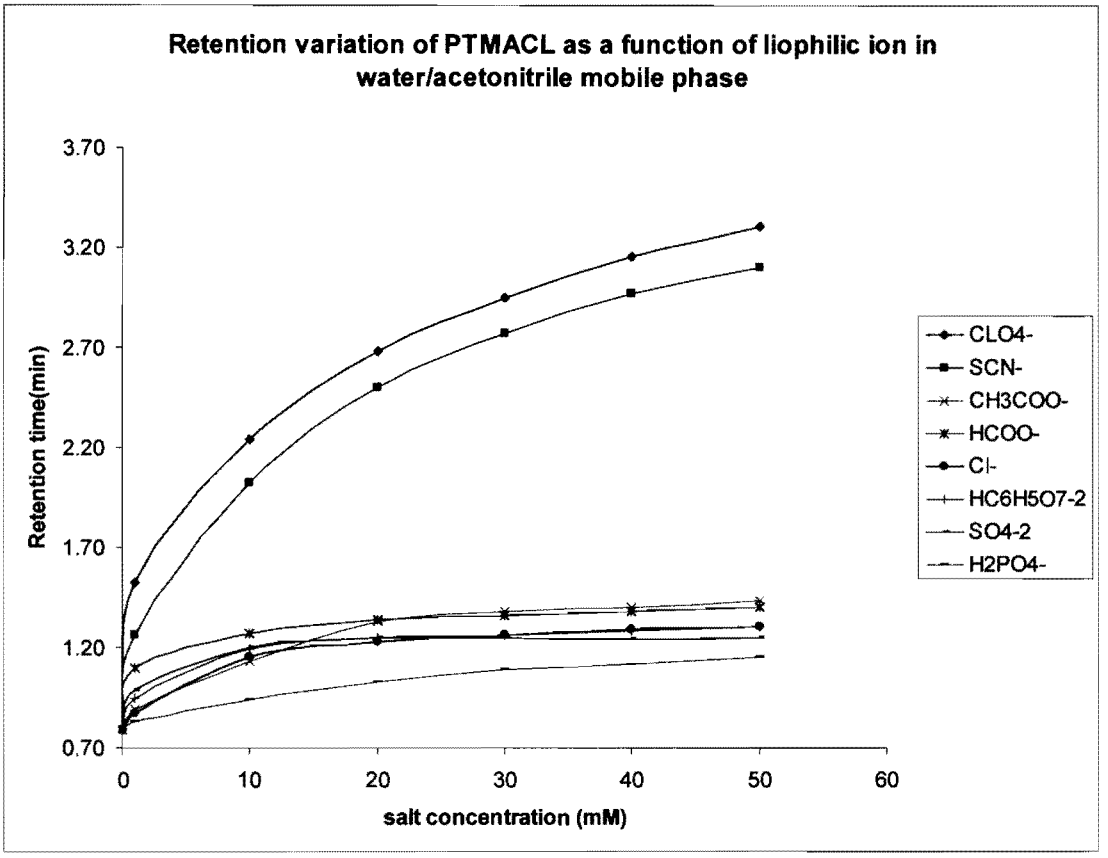


Figure 12. Variation of PTMA+ retention factor as a function of varios counterion concentrations. Column and conditions are the same as in Figure 11, data were not optimized using mathcad.

5.3-Evaluation of basic analyte retention as a function of liophilic salt concentration without adjusting mobile phase pH.

An ionic analyte in water/organic solution is in solvated form with highly hydrophilic solvation shell, this increase in hydrophilicity reduces the analyte retention in reversed-phase HPLC. When a liophilic ion is introduced in the mobile phase, this ion disrupts the analyte solvation shell and increases its hydrophobicity, which results in increasing the analyte retention time[21].

Experimental results of the retention time of aniline and pyridine without pH control in the mobile phase are shown in Figure 13.and Figure 14.

Mobile phases were prepared by adding liophilic ions with concentrations ranging in between 1mM and 100 mM to a solution of 95% water and 5% acetonitrile. The pH's of the solutions were measured at each concentration of liophilic ion introduced to the aqueous portion of the mobile phase, Figure 15 shows the pH of different aqueous mobile phase portions at each liophilic ion concentration.

Analyte retention correlates with the variation of pH and salt addition in the mobile phase. Generally, basic analyte retention is negligible when a salt is dissolved in mobile phase with pH 2 units above the analyte's pka.

As shown in Figure 13 and Figure 14 the addition of liophilic ions to the mobile phase when the pH in not controlled, shows no significant difference in the retention of the analytes.

The retention time of aniline and pyridine follows the same pattern as the pH in solution due to the addition of liophilic ion to the mobile phase.

When aniline and pyridine are close to their pKa's, 4.6 and 5.2 respectively, these analytes are presented in the mobile phase in two forms in equilibrium displaying a secondary effect. These two forms of analyte show different affinity to the stationary phase. These different affinities to the mobile phase impact the analyte retention time and the peak symmetry.

When the pH of the mobile phase is not buffered to an acidic pH (where the bases are in the fully protonated form), a partial protonation leads to a coexistence of two analyte forms and the retention process is a superposition of ionization and adsorption processes.

The addition of liophilic ions to the mobile phase increases the ionic strength thus facilitating ionic equilibrium making peaks of not fully protonated analytes more symmetrical while protonated analytes increases their retention.

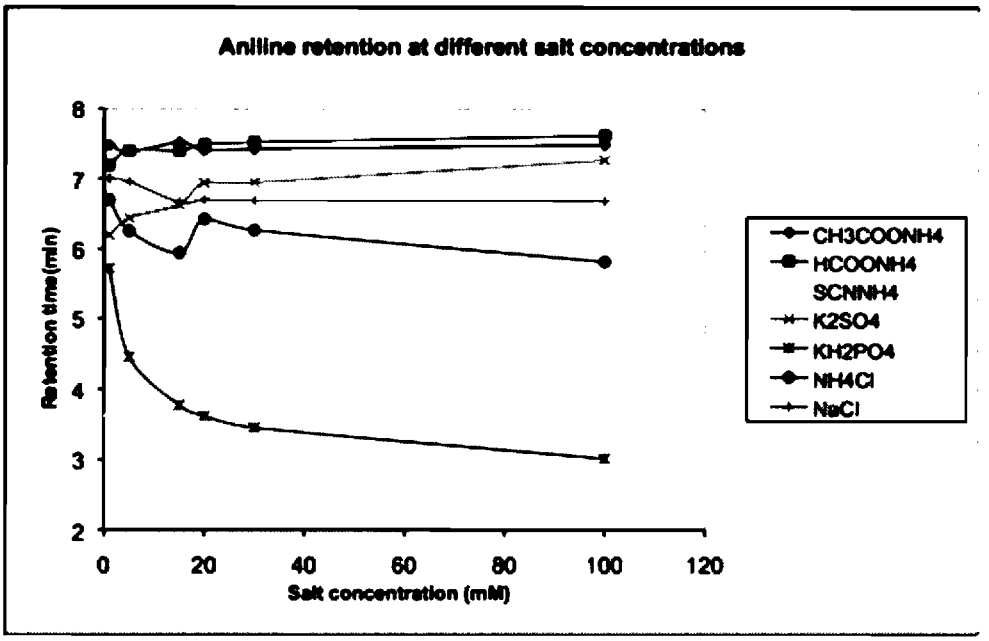


Figure 13. Aniline retention was evaluated on a YMC-Pack Pro C18, AS-300-3,S-3μm, 12nm, 50X4.6mm ID, S/N 040511295 column,experiment run at room temperature, salt was added to the aqueous portion of the mobile phase.

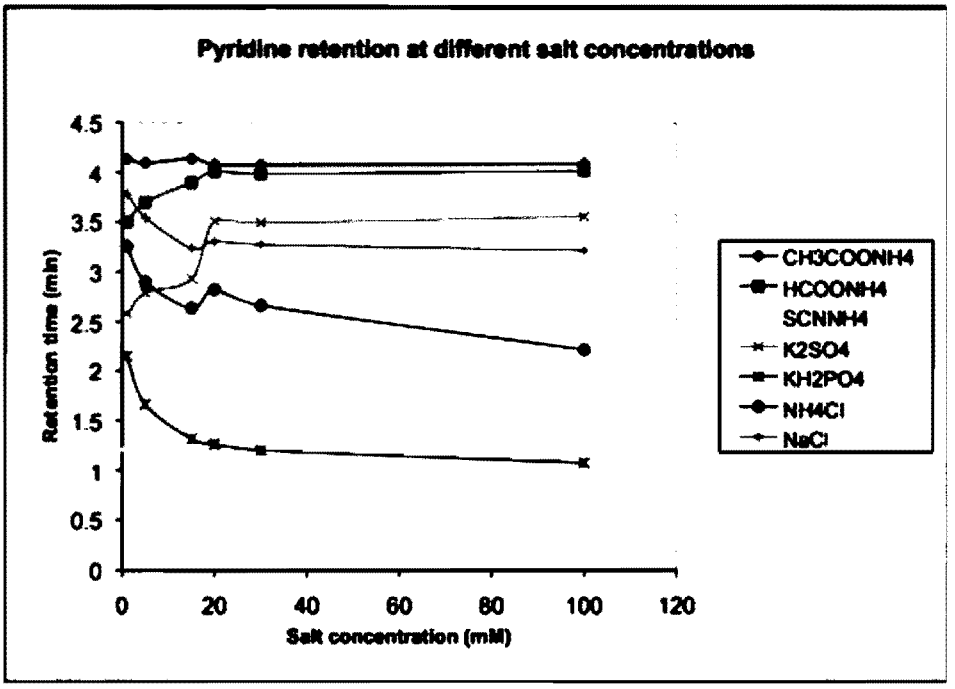


Figure 14. Pyridine retention was evaluated on a YMC-Pack Pro C18, AS-300-3,S-3µm, 12nm, 50X4.6mm ID, S/N 040511295 column, experiment run at room temperature, salt was added to the aqueous portion of the mobile phase.

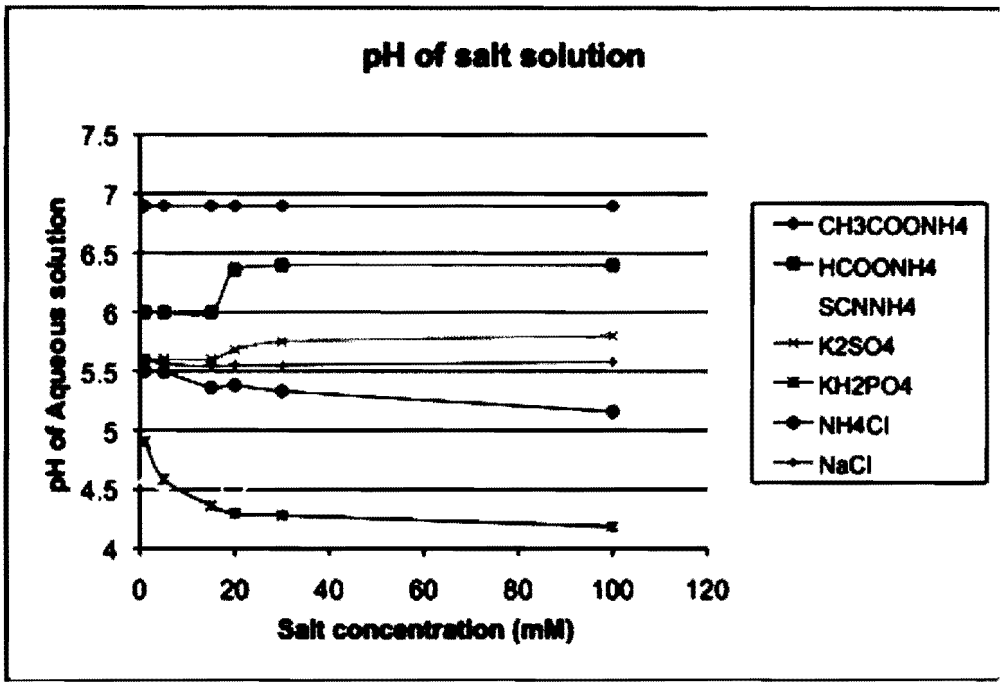


Figure 15. Aniline retention was evaluated on a YMC-Pack Pro C18, AS-300-3,S-3μm, 12nm, 50X4.6mm ID, S/N 040511295 column, experiment run at room temperature, salt was added to the aqueous portion of the mobile phase.

5.4-Evaluation of basic analyte retention as a function of liophilic salt concentration adjusting mobile phase pH.

Experiments discussed previously were performed without any pH adjustments and without any additional variations of the mobile phase ionic strength. These conditions were intentionally selected to avoid the influence of the ionic strength discussed by Stahlberg[89] or pH variations. Selected analyte ionization is independent on the mobile phase pH. On the other hand it is known that at very low ionic strength (usually below 10 mM) significant erratic variations of ionic analyte retention could be observed, usually this is associated with high statistical charge distribution fluctuations in water-organic mixtures with low dielectric constant.

We also measured the retention of other ionizable model analytes at controlled pH and ionic strength conditions.

Aqueous pH 3 was selected to ensure complete ionization of aniline and pyridine, corresponding pK_a's are 4.6 and 5.2. Since all these studies were performed at low organic content, 5% of acetonitrile did not cause the pH and pKa shift and did not affect the analyte ionization state. pH was adjusted by gradual addition of HCl solution to the aqueous portion of mobile phase with simultaneous monitoring the solution pH. In both cases for aniline and pyridine only the addition of thiocyanate salt demonstrated very strong effect as seen in Figure 16 and Figure 17, although all other ions did show definite

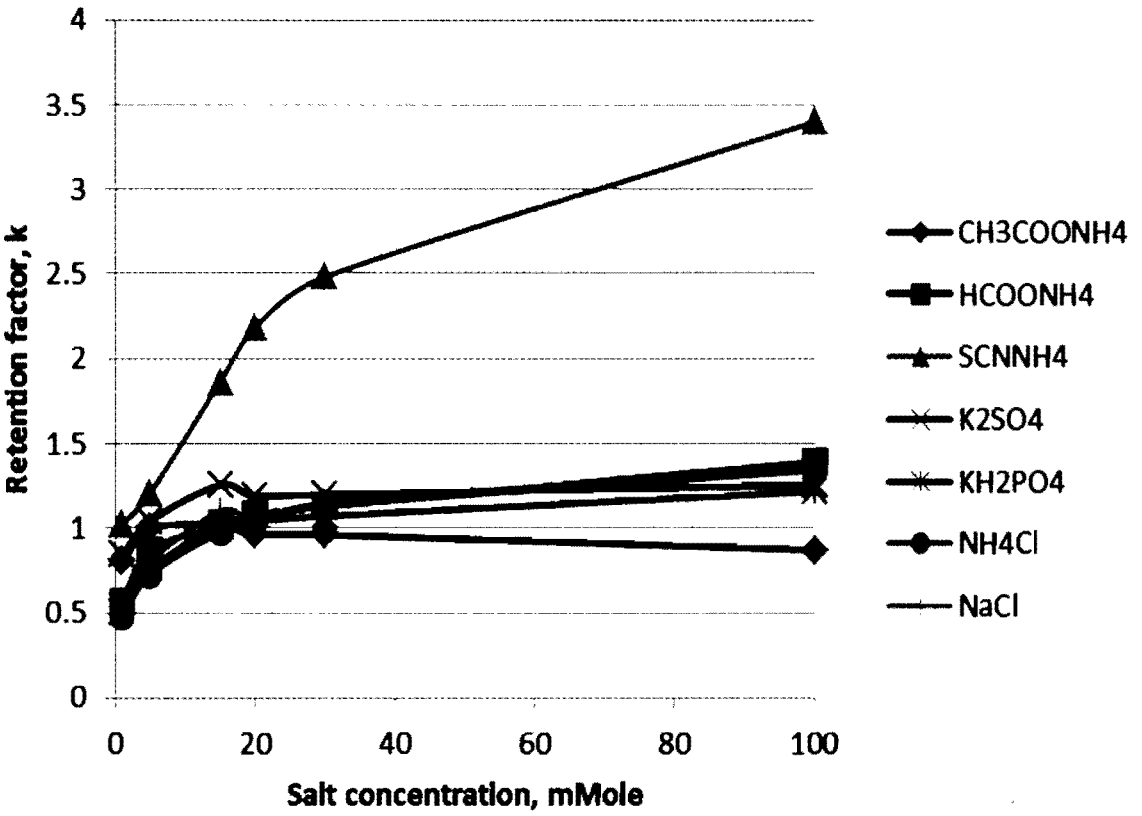


Figure 16. Aniline retention variation with addition of different counterions into the mobile phase at pH 3 of 5/95 acetonitrile water.

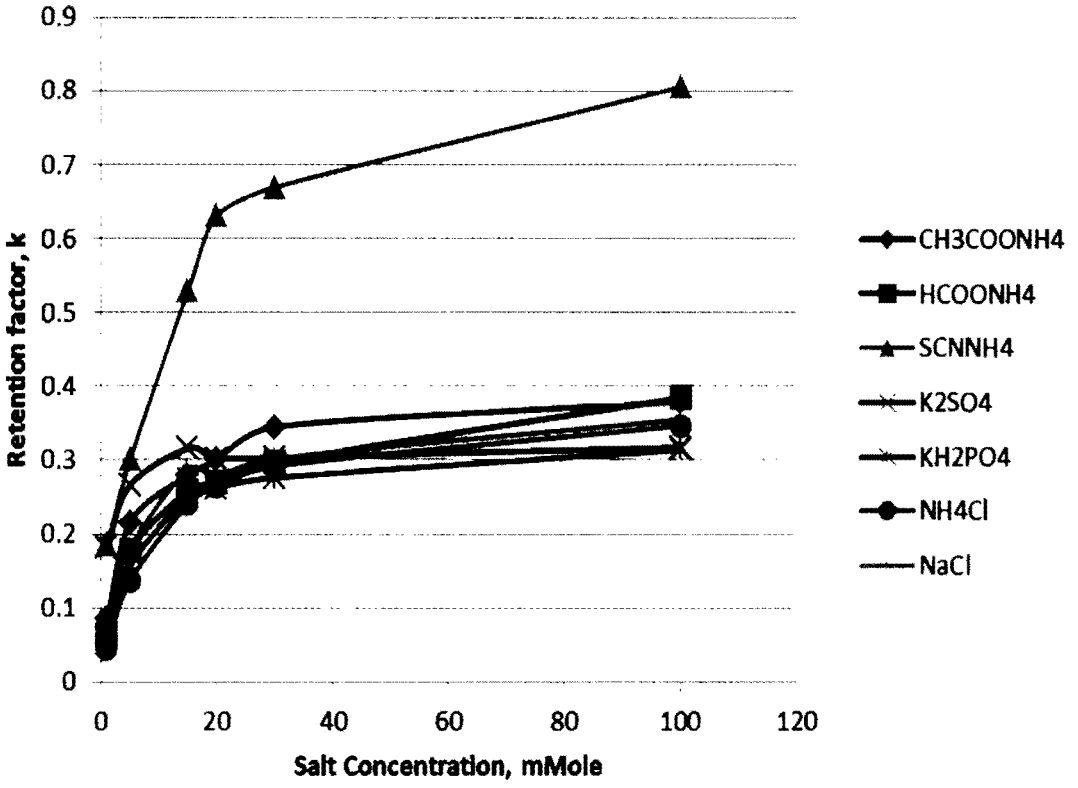


Figure 17. Pyiridine retention variation with addition of different counterions into the mobile phase at pH 3 of 5/95 acetonitrile water.

chaotropic behavior with very little difference between each other. It is practically impossible to determine any specific sequence in the strength of their influence on the retention of either aniline or pyridine, while there is definitely no sign of any kosmotropic influence either.

5.5-Discrepancy in the order of ions study with Hofmeister series

Our results prove to show a discrepancy in which salts are organized in the Hofmeister series. This discrepancy on retention possibly can occur because HPLC retention is a sum multiple effects and not just a single effect such as viscosity.

Variations in the eluent composition for example, can cause changes in the mobile phase dielectric constant and this change can affect the strength of the ionic interactions[90]. As the organic concentration increases the dielectric constant of the medium is decrease, this provides a bigger tendency for ionic interaction between the liophilic ion and the protonated basic analyte.

The stability of ion-association complexes species is favored by mobile phases with low dielectric constant. As it is shown in equation 8, lower dielectric constants produce greater attraction forces between oppositely charged species.

$$F = \frac{q_1 q_2}{3\pi D^2 \varepsilon} \qquad 8$$

Ions are placed in the Hofmeister series based on their "chaotropic" and "kosmotropic" properties.

Hofmeister series organize ions according to their influence on the solvation shell, either facilitating water structurization (kosmotropic effect) or destabilization structural arrangement of water molecules (chaotropic effect)[91,92].

The mechanism of Hofmeister effects has been extensively investigated and debated. These studies have led to comprehensive theories of aqueous electrolyte solutions which can explain numerous physicochemical properties of these solutions but still be unsuccessful to completely explain the mechanism of salt effects on molecules or why these effects follow the Hofmeister series[93,94,95,96,97,98,99]. Particular attention has been paid recently to the role of dispersion forces[100] , but historically the most difficult aspect of explaining salt effects in solution has been the consideration of how the ions change the nature of water hydrogen bonding[101] .

There is extensive data supporting the idea that ions have significant impact on local water hydrogen bonding behavior[102,103,104,105,106,107,108,109,110,111]. One theoretical model, developed by Marcus, has been able to explain quite well for a number of thermodynamic parameters of a variety of ions in solution through modeling ion effects on the first hydration layer. Salt effects on water have been most commonly described in terms of the chaotropicity or kosmotropicity of the solutes. Possibly the best description of the chaotrope/

kosmotrope description is the one offered by Collins: His description mentions that binding of chaotropes to water molecules is weaker than binding of water molecules to each other; and binding of kosmotropes to water is stronger than binding of water molecules to each other. Chaotropicity appears to be related to low charge density, consequently large singly-charged ions tend to be chaotropic. Their low charge density means that they have smaller effects on the local hydrogen bonding. Kosmotropicity, on the other hand, is associated with a high charge density, therefore small or multiply-charged ions tend to be kosmotropic. The high charge density of small charged ions means that they interfere strongly with local water hydrogen bonding.

The position of ions in the Hofmeister order has been shown to correlate with the Jones–Dole viscosity B coefficient, equation 9.

Individual ions may be systematically classified as chaotropes or kosmotropes by the sign of the Jones-Dole viscosity B coefficient (negative and positive, respectively), as shown by the pioneering work of Cox and Wolfenden (1934), which was later extended by Kaminsky (1957), Stokes, and others (Robinson et al., 1981). The viscosity B coefficient correlates with charge density and is defined by the expression

$$\frac{n}{n_0} = 1 + AC^{1/2} + BC \qquad 9$$

Where η is the viscosity of an aqueous solution of ions, η_0 is the viscosity of water at the same temperature, C is the salt concentration, A is an electrostatic

term, and B is a measure of the strength of ion– water interactions . The viscosity B coefficient, like chaotropicity/ kosmotropicity, has been shown to correlate with charge density. The correlation between viscosity effects, charge density, and Hofmeister order suggests a causal relationship between the character of ions and their effect on solvation properties through impact on HPLC ionic analyte retention.Viscosity B coefficients for relevant ions are given in Table 2. This table shows ions classified as chaotropes or kosmotropes depending on sign from negative for chaotropes to positive for kosmotropes. A graphic representation of the classification of ions as kosmotropes or chaotropes based on viscosity values can be observed in **Figure 18**. Chaotropicity of ions studied based on B-coefficient. It was our intention to study systematically the effects of salt addition using kosmotropic and chaotropic salts on the retention of ionic and ionizable basic analytes.In HPLC practically only chaotropic ions were studied and the effect of disruption of solvation shell was associated with the increase of analyte retention due to facilitating the ability for hydrophobic interactions with stationary phase after disruption of solvation shell[112]. It is logical to assume that ions facilitating water structurization and thus strengthening ionic analyte solvation should decrease the retention in reversed-phase HPLC, although this effect to our knowledge has not been reported yet.Cumulative Hofmeister series for anions composed from different sources [70, 51, 72] could be represented by the following sequence: CO_3^{2-} ; SO_4^{2-} ; $S_2O_3^{2-}$; $H_2PO_4^-$; $HCOO^-$; $CH_3SO_3^-$; F^- ; Cl^- ; Br^- ; NO_3^- ; I^- ; CH_3COO^- ; CF_3COO^- ; BF_4^- ; ClO_4^- ; SCN^- ; PF_6^-

ION	B- coefficient
SCN-	-0.103
CLO4-	-0.061
NO3-	-0.046
Cl-	-0.005
HCOO-	0.052
SO4-2	0.206
CH3COO-	0.246
H2PO4-	0.340

Table 2. Jones-Dole viscosity B coefficient for ions studied

Order of chaotropicity based on Viscocity coefficient value.

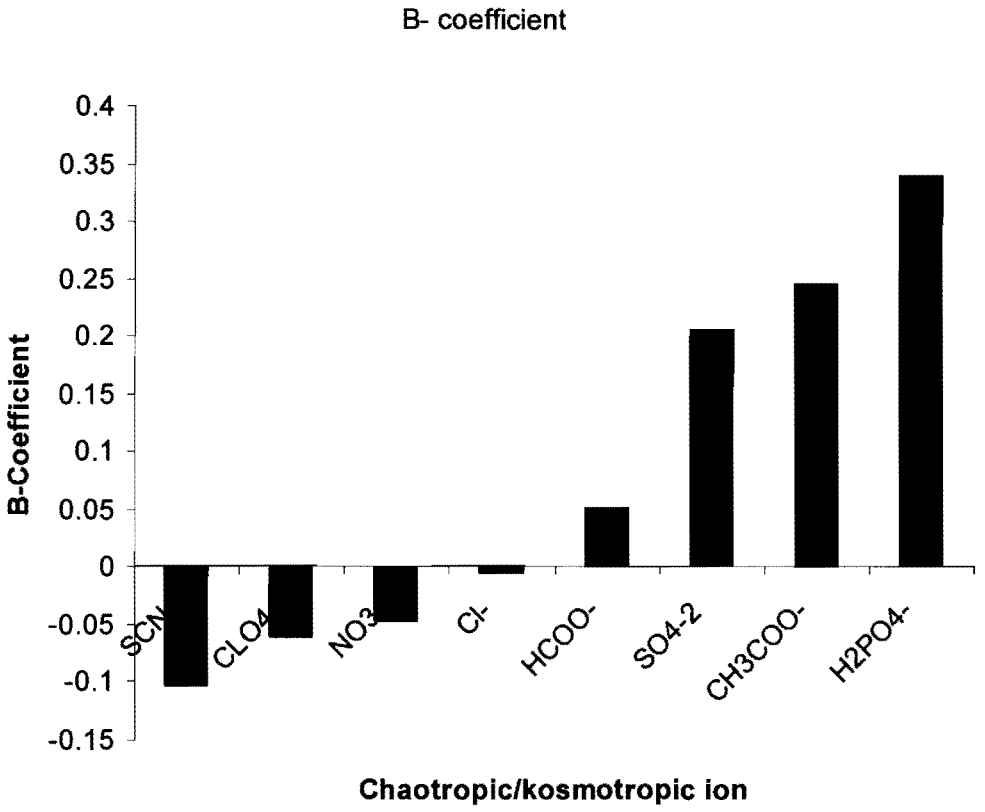


Figure 18. Chaotropicity of ions studied based on B-coefficient.

Where the leftmost ions are the most kosmotropic and the rightmost are the most chaotropic accordingly. Usually F^- or Cl^- is considered to be the neutral in terms of chaotropic or kosmotropic properties.

As shown previously, we only selected a subset of ions from the sequence shown above. In our study we use SO_4^{2-} ; $H_2PO_4^-$; $HCOO^-$; F^- ; Cl^- ; Br^- ; NO_3^-; I^-; CH_3COO^-; CLO_4^-; SCN^-. Although preliminary experiments demonstrated that all halogens show very minor difference between each other and for all studies we use just Cl^- ion.

As can be seen in table 3, there is a remarkable difference between the order of the ions from the Hofmeister series and the order of the same ions based on chromatographic measurements. This difference in the order could be assigned to the complex retention mechanism in HPLC. One possible contribution to the retention mechanism of protonated basic analytes is the formation of a stable ion association complex due to the character of the chaotropic ion added to the mobile phase, a highly hydrated ion such $H_2PO_4^-$ has the least ability to form an ion-complex and on the other hand a poorly hydrated ion such as ClO_4^- has the highest ability to form an ion-complex and therefore the highest retention time in acetonitrile. An apparent difference in elution when using acetonitrile and methanol is observed in the case of poorly hydrated ions such $ClO4^-$ and SCN^- , this difference in the order of elution could be attributed to the nature of the organic modifier since alcohols such methanol, are considered to be less structured, they lose more entropy than water when immobilized near the ion, hence the larger apparent solvation

Table displaying the discrepancy on the Hofmeister series with our experimental results.

B- coefficient	Methanol	Acetonitrile
SCN^-	SCN^-	CLO_4^-
$CLO4^-$	CLO_4^-	SCN^-
Cl^-	CH_3COO-	CH_3COO^-
$HCOO^-$	$HCOO^-$	$HCOO^-$
SO_4^{-2}	Cl^-	Cl^-
CH_3COO^-	SO_4^{-2}	SO_4^{-2}
$H_2PO_4^-$	$H_2PO_4^-$	$H_2PO_4^-$

Table 3. Showing discrepancies in chaotropic/kosmotropic salts in the Hofmeister series.

numbers. An electrostatic field effect on the entropy of solvation is not related to the strength of any bonding between the ion and the solvent, but only to the number of molecules affected. Standard molar entropy of transfer for ClO_4^- from water to acetonitrile seems to be greater SCN^- than water to methanol, therefore giving a greater electrostatic component on the adsorbed organic layer for the retention mechanism.

5.6-Effect of analyte size on retention

Ion hydration is strongly dependent upon ion surface, charge density and progressing from strong hydration for small ions of high charge density (kosmotropes) to weak hydration for large monovalent ions of low charge density (chaotropes). It is useful to consider an ion to be a sphere with a point charge at the center. As the sphere become larger, the water molecules at the surface of the sphere becomes further from the point charge at the center of the sphere.

When the water molecules at the surface of the sphere are so far from the point charge at the center that water–ion interactions are weaker than water–water interactions in bulk solution, the ion is a chaotrope.

In the case of ammonium quaternary, figure 19, it is expected that the chaotropicity increases with the size of the molecule in the following order:

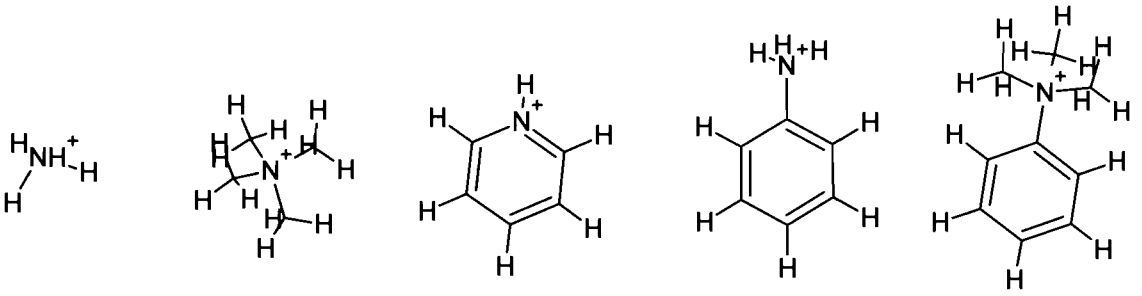


Figure 19. Ammonium quaternary compounds

A solution consisting of ions of a variety of sizes will be likely to separate according to size. Small ions of opposite sign and comparable size will tend to pair since they form stronger interactions than those between small and large ion complex. The large ions of opposite sign and similar size will also be likely to pair because their formation releases water for formation of stronger water-water interactions.

Ion size controls the tendency of appositively charged ions to form inner sphere ion pairs. Small ions of opposite sign spontaneously form inner sphere ion pairs in aqueous solution. Large ions of opposite sign spontaneously form inner sphere ion pairs in aqueous solution.

The characteristic size (see Figure 20 for approximate molecular radius) for Pyridinium, Anilinium and PTMA+ will give them the tendency to form inner sphere ion pairs with chaotropic ions such as SCN^- or $CLO4^-$.

Mismatched ions of opposite sign do not spontaneously form inner sphere ion pairs in aqueous solution. A large mono-valent cation that has a radius larger than 1.06Å is considered to be chaotropic; a large mono valent anion that has a radius larger than 1.78 Å is considered to be chaotropic. All the mono valent

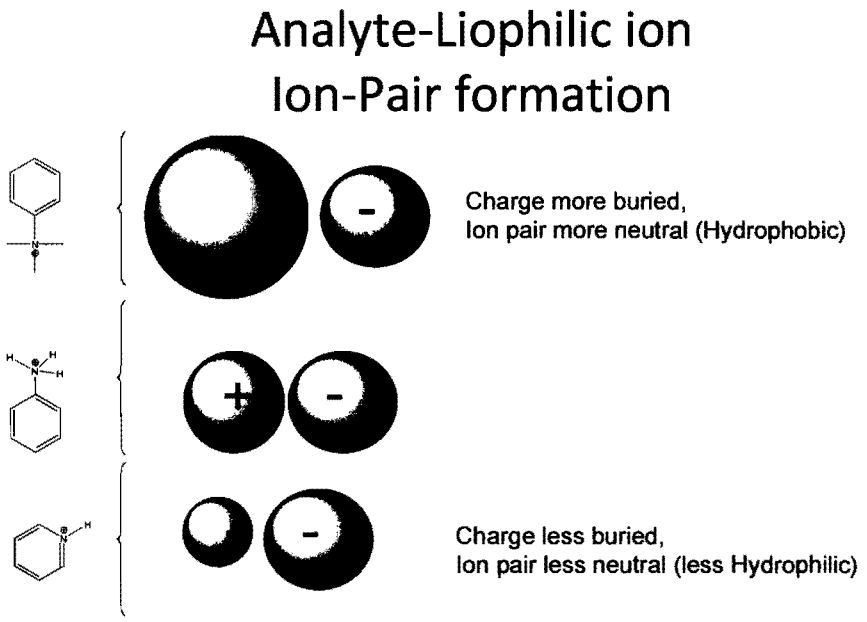


Figure 20. Effect of size on analyte retention

Approximate molecular radius for analytes in our study are:

Pyridinium: 4.99Å

Anilinium: 5.29 Å

$PTMA^+$: 7.09 Å

anions in our study have a radius greater than 1.78 Å, with chloride being in the border line. Sulfate on the other hand, is a di-valent anion and form hydration shell from small rings of hydrogen bonded water. This anion will form a solvent shared ion-pair.

As can be seen in Figure 21, the size of the analytes, pyridinium, anilinium and phenyltrimethylammonium ($PTMA^+$) can possible affect the formation of three different inner sphere ion pairs with increase of hydrophobicity as the size of the analyte increases. This increase in hydrophobicity due to the increase of analyte size is reflected in increase of retention factor.

5.7-Effect of organic modifier on analyte retention.

Acetonitrile is a polar solvent, miscible with water in all proportions but, nevertheless, has sufficient dispersive (hydrophobic) properties to elute substances from a liquid chromatography column by dispersive interactions with the solute. Starting with pure water as the mobile phase and progressively adding acetonitrile to the water, the mobile phase is made progressively more dispersive in character and progressively elutes more dispersive substances. When acetonitrile/water is used as mobile phase, acetonitrile forms a thick adsorbed layer on the surface of the stationary phase providing a suitable media for the adsorption of liophilic ions. Acetonitrile

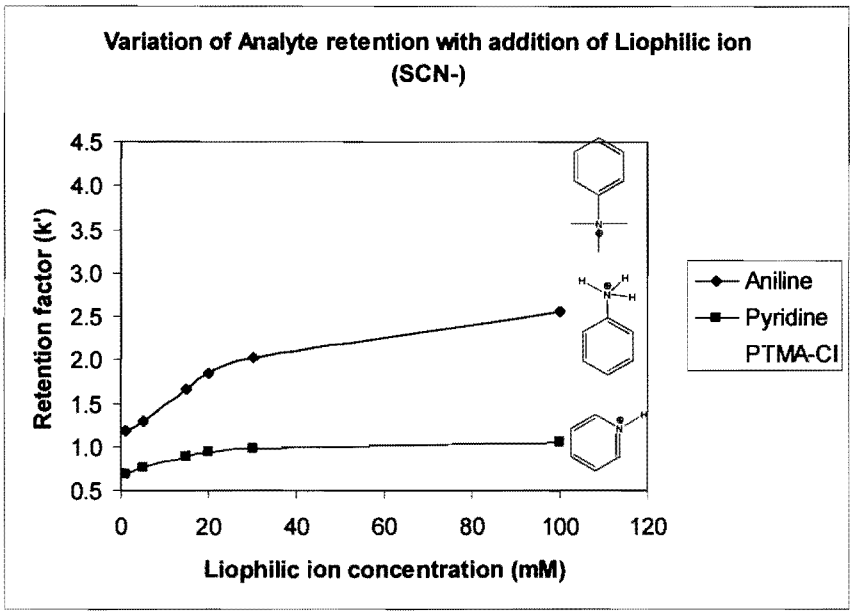


Figure 21. Analyte retention as a function of chaotropic analyte concentration in acetonitrile/water mobile phase.

cannot form hydrogen bonds therefore it does not participate in the solvation of analytes. Methanol on the other hand, forms a strong associate with water so that at high concentrations of water, the mobile phase behaves as a binary mixture of water and water-methanol associate.

Opposed to acetonitrile-water mixtures the complex nature of methanol-water mixtures makes solute retention more difficult to predict from the original methanol content of the mobile phase.

Methanol is a protic solvent and participates in inclusion of methanol molecules in the analyte solvation shell. This inclusion of methanol molecules in the analyte solvation shell might promote a distortion in the analyte's peak shape as can be seem in Figure 22, this peak shape distortion is not seen in when acetonitrile is used instead of methanol as can be observed in Figure 23.

All our previous discussed studies were performed in acetonitrile/water mixtures and as mentioned in the literature, acetonitrile being very pi-electron rich molecule and forming the adsorbed layer of significant thickness on the surface of the stationary phase creates favorable conditions for the actual retention of liophilic ions with significant delocalization of electrons. This effect creates additional retaining conditions for ionic analytes.

Comparison of retention time and peak shape for $PTMA^+$ retention as a function of chaotropic salt in Water/Methanol mobile phase.

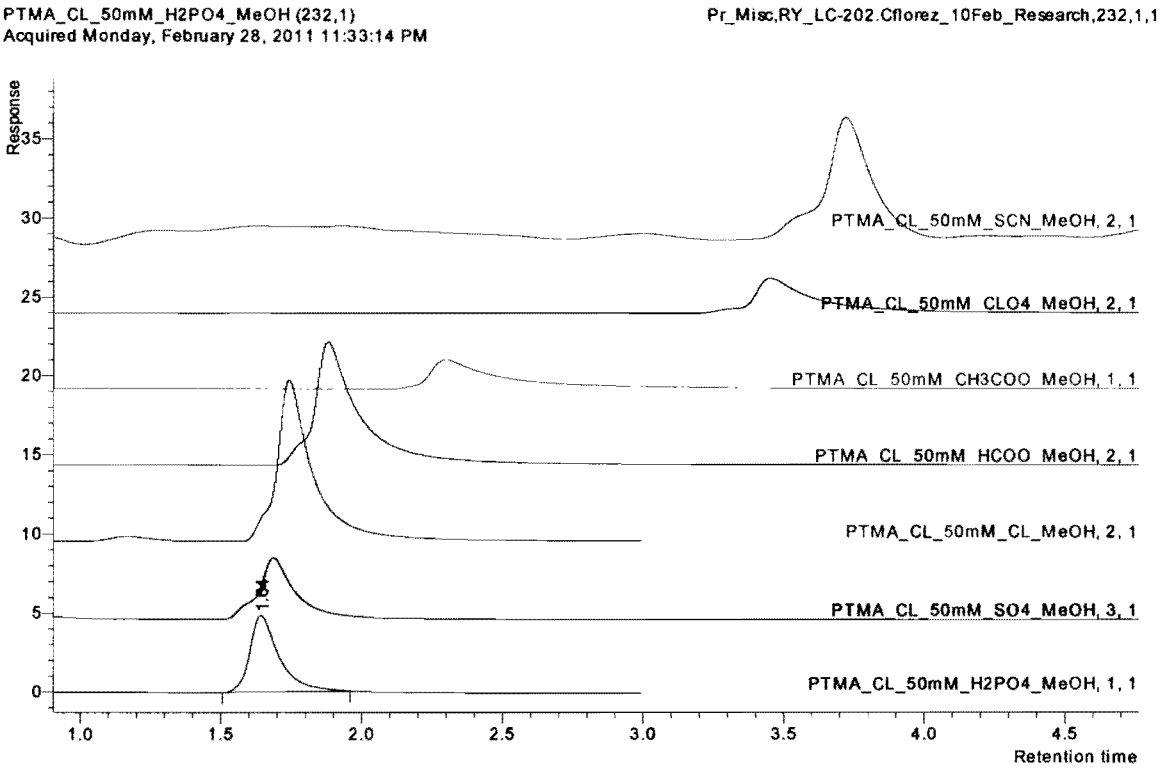


Figure 22 Variation of analyte retention time as a function of salt concentration in water/methanol mobile phase

Comparison of retention time and peak shape for $PTMA^+$ retention as a function of chaotropic salt in Water/Acetonitrile mobile phase.

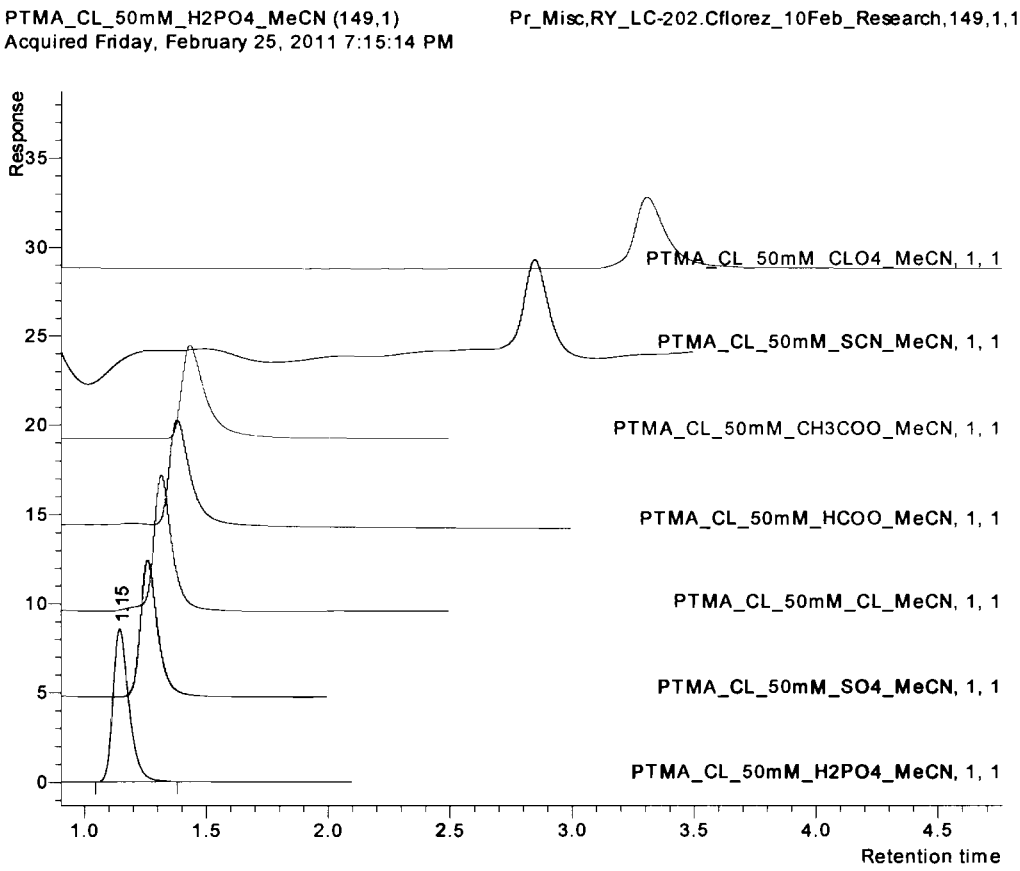


Figure 23. Variation of analyte retention time as a function of salt concentration in water/acetonitrile mobile phase

We studied the retention of phenyl trimethyl amine in methanol/water mixtures as a function of the amount of added counterions. As it could be seen in Figure 24 the retention of $PTMA^+$ increases with the increase of the counterion concentration, so this means that the chaotropic effect could be observed in methanol/water eluent also.

These experimantal data also were optimized with complex parameteric minimisation to verify their fit to the equation 7. Figure 25 shows the optimized representation using the equation. Corresponding parameters of this equation for each curve are shown in Table 3.

In this case also the expected most kosmotropic ion demonstrate fastest chaotropic effect, or in other words the transfer from low retention ("most solvated state", k_2) to the most desolvated state, (k_1) requires the addition of just 5 mM of ammonium sulfate as it could be seen in Figure 25 where these experimental data are shown together with optimization curves.

Chloride and thicyanate require more than 15 mM of salt added to the mobile phase to reach retention shift saturation state, when further salt addition did not affect analyte retention, while for sulfate, the ion with the most localized electrons, this saturation is achieved at already 5 mM concentration. Overall influence of chloride and sulfate is approximately the same.

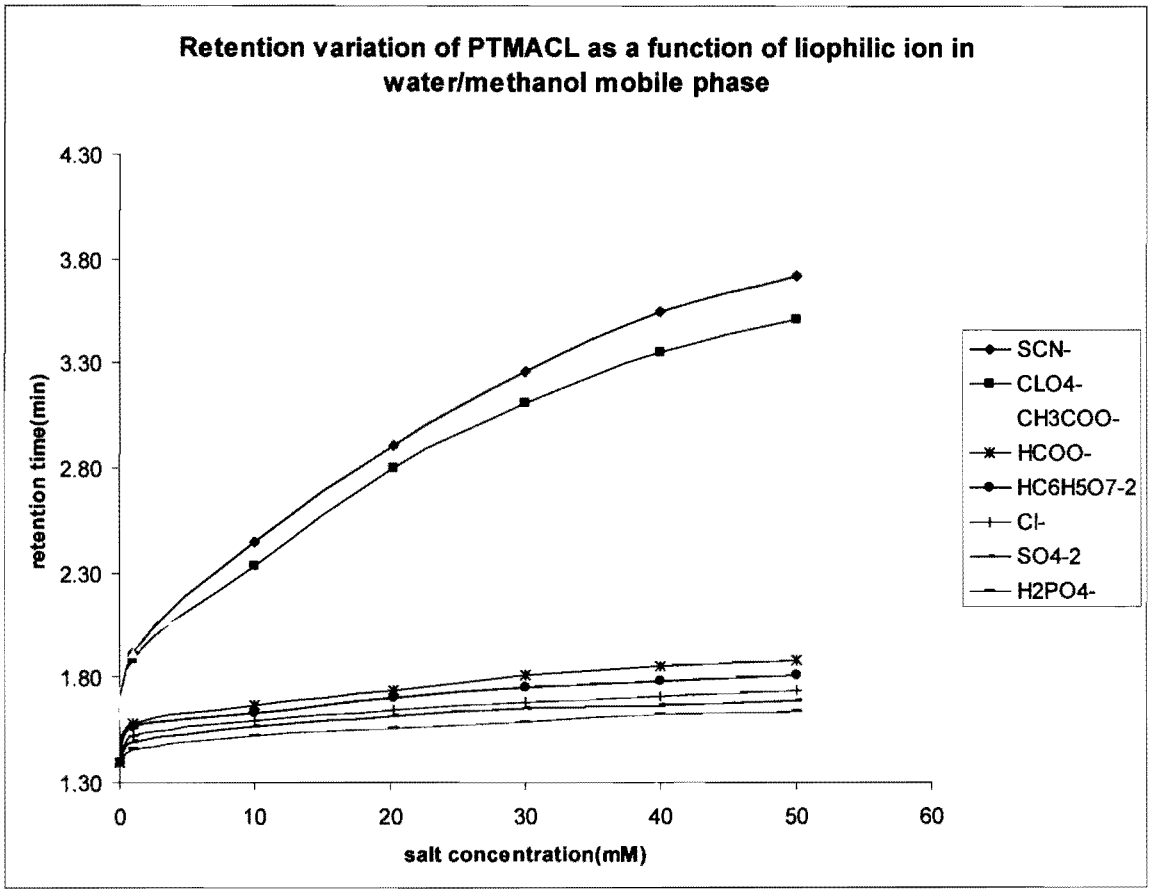


Figure 24. Variation of PTMA+ retention factor as a function of varios counterion concentrations. Column and conditions are the same as in Fig. 22, data were not optimized using mathcad.

Graph comparing the effect of addition of the most kosmotropic ion SO_4^{-2}, the intermedium Cl^-, and the most chaotropic ion SCN^- on the retention of $PTMA^+$.Data optimized using MathCad

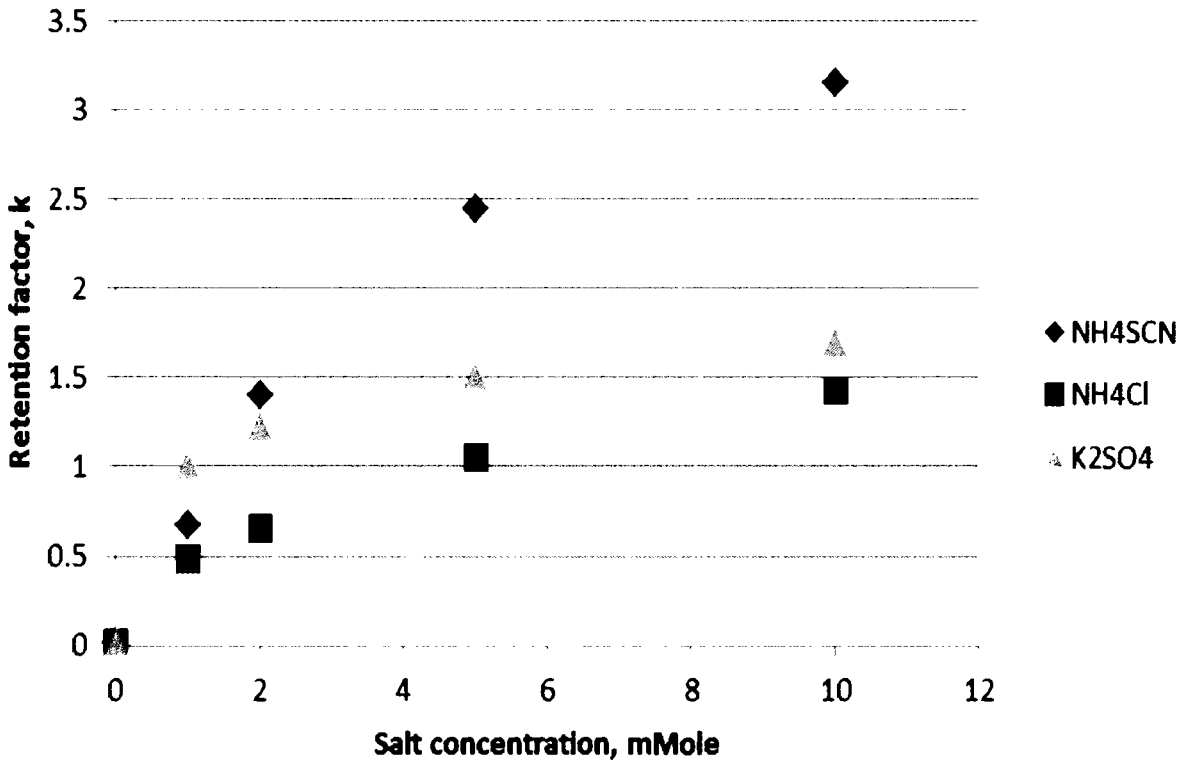


Figure 25. The effect of chloride Cl^-, sulfate SO_4^{-2} and thiocyanate SCN^- salts on the retention of phenyl trimethyl ammonium ($PTMA^+$) on YMC Pro-Pack C18 column, mobile phase 5% of methanol in water.Graph comparing the most kosmotropic ion SO_4^{-2}, the intermedium Cl^-, and the most chaotropic ion SCN^-.

Table 4. Limiting retention factors and desolvation constant for chaotropic effect dependence of PTMA on different salt concentration on methanol/water mixture.

	SCN^-	Cl^-	SO_4^{2-}
k_1	4.777	1.959	1.798
k_2	-0.028	0.043	0.021
K	0.203	0.245	1.137

Even though some previous data indicate that methanol is not a suitable solvent for the chaotropic effect, we demonstrated that a chaotropic effect is in fact presented when liophilic ions are added into methanol/water mobile phases.

Ion-pairing agents could affect the retention of both acidic and basic compounds, while chatropic ions are used for improving the retention of only basic analytes. Increasing retention of liophilic ions was seen from the increasing of acetonitrile concentration as well as methanol. In Figure 10, it can be observed the thicker adsorbed layer for acetonitrile at 5% organic modifier used during the experiments, as stated previously, acetonitrile forms a thick adsorbed layer on the surface of hydrophobic bonded phase, while methanol forms a classical monomolecular adsorbed layer. The thick adsorbed layer of acetonitrile acts as a pseudo-stationary phase and allows adsorption of chaotropic ions on this layer. The pseudo-stationary phase is suitable for ion accumulation, this creates an electrostatic potential on the stationary phase surface resulting to enhancement of the retention of protonated basic analytes. However, the increased retention with the increase in organic solvent for reversed-phase HPLC, is seen in low concentration of acetonitrile (0-20%). At high acetonitrile concentration, more than 25%, the retention of basic analytes starts to decrease due to the normal effect of the increase of organic composition in the mobile phase.

5.8-Effect of temperature on the retention of basic protonated analytes as a function of liophilic ions in the mobile phase.

In our study, the retention of aniline was evaluated as a function of acetonitrile concentration with 20mM of ammonium thiocyanate between 5°C and 50°C. It is interesting to notice (Figure 26) that the effect of temperature on analyte retention is more pronounced at lower concentration of organic modifier with almost not effect at all when the concentration of organic modifier in the mobile phase is higher than 16%.

Temperature and pressure both have effects on the kosmotropic/chaotropic status with the effects disappearing at high temperatures, particularly at high concentrations[113] For example, at very high pressures (0.6 GPa) Na^+ ions change from being weak kosmotropes into weak chaotropes as their links to water molecules are preferably broken.

Some of the variables affected with change in temperature are: ionization of solutes, ionization of buffer components and value of thermodynamic equilibrium constant.

It is known that increase in temperature decrease the dielectric constant due to the difficulty of water dipoles to shield electrical charges because of higher kinetic energy. This decrease of the dielectric constant of water with increasing temperature, lower polarity and disrupt the hydrogen, these changes in water may alter $\Delta H°$ and $\Delta S°$. Also at high temperatures, hydrogen

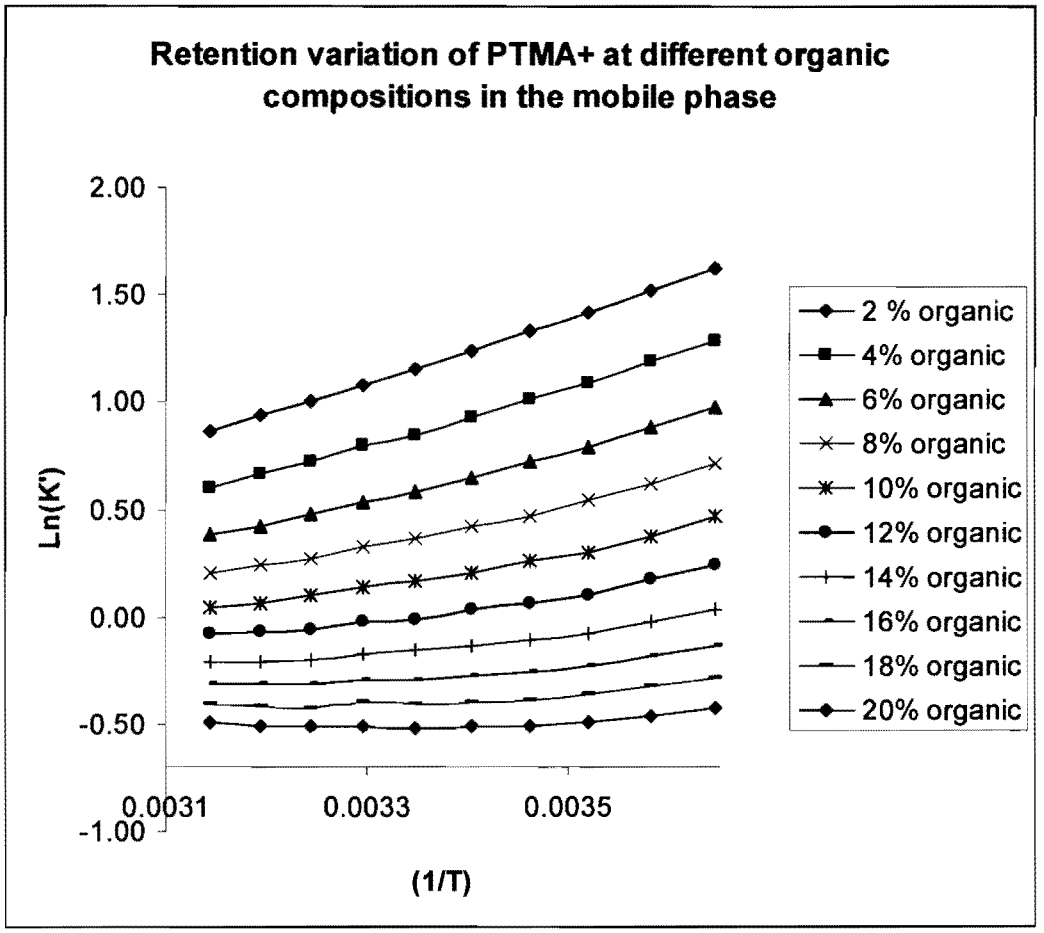


Figure 26. Retention variation for Phenyl trimethyl ammonium as a function of temperature at different organic modifier concentrations.

bonding and solute hydration are weaker. The entropy change due to the release of hydrophobically bound water molecules upon transfer of the solute from mobile phase to the stationary phase is lower, hence $\Delta S°$ is expected to decrease and not remain constant.

The solvent strengths of all mixtures change with temperature and this influence the selectivity [114].

The retention time dependency with temperature for ion-pair species, is much more complicated. Retention results from at least four independent equilibria, each characterized by its own $\Delta H°$ and $\Delta S°$.

6-Conclusions

This study show:

1) That the addition of practically any inorganic counterion in the mobile phase will result in at least slight increase of the retention of ionic and ionizable analytes. The variation of the degree of influence is related to the counterion liophilicity or its ability to hydrophobic interaction. This usually increases with the increase of the electron delocalization on selected ion and with the increase of ion polarizability. For example, the most chaotropic inorganic anion known today is PF_6^-. This ion features spherical symmetry with high density of all fluorine valence electrons which facilitates such significant electron delocalization, this small ion demonstrates significant hydrophobic properties. Ammonium hexafluorophosphate salt is soluble in pure acetonitrile up to 1 molar concentration. No other inorganic salt could be dissolved in acetonitrile to any noticeable concentration. Other anions with strong charge delocalization are ClO_4^-, BF_4^-, SCN^-, CF_3COO^-, and they all demonstrate significant chaotropic properties, or shall we simply say "cause increase of charged analyte retention", since word "chaotropic" indicate the mechanism of this retention increase as destabilization and destruction of water solvation shell, and also assume the existence of the opposite process –

"kosmotropic effect", which supposed to decrease the retention of charged analytes.

2) We were not able to find any sign of retention decrease while using "kosmotropic" counterions as mobile phase additives. Which is an indication of either nonexistence of this kosmotropic effect in HPLC, or it is an indication that overall influence of mobile phase ionic additives is much more complex than just disruption of analyte solvation shell. This solvation shell disruption probably happens just with increase of the mobile phase ionic strength above 10 mM, the limit generally noted as sufficient for stabilization of erratic retention of ionic or ionizable analytes in un-buffered mobile phases.

7-List of abbreviations

pH	Measure of the acidity or basicity of an aqueous solution
pKa	The negative logarithm of the acid dissociation constant
BH^+	Protonated base
B	Neutral base
K_a	Acid dissociation constant for the base
K_0	Retention factor of neutral base
K_1	Retention factor protonated base
K_A	Thermodynamic association constant
N	Avogadro's number
W+-(r)	Anion-cation pair potential
D	Critical distance needed for ion-pair formation
Z	Ionic charge
e	Electron charge
ε	Dielectric constant
k	Boltzmann's constant(in the Bjerrum equation)
q	Distance for which the probability of finding a counter ion in a spherical shell next to the central oppositely changed ion is minimum.
$A^+\|\|B^-$	Solvent separated Ion-pair
A^+B^-	Contact ion-pair

ΔH	Standard enthalpy for the analyte adsorption on the stationary phase
ΔS	Standard enthalpy for the analyte adsorption on the stationary phase
R	Gas constant
T	Temperature
Φ	Ratio of the surface area of the column to the mobile phase volume
V_o	Void volume
K'	Retention factor.
η	Viscosity of an aqueous solution of ions,
η_0	Viscosity of water at the same temperature
C	Salt concentration
A	Electrostatic term
B	Measure of the strength of ion– water interactions
F	Attraction force between appositively charged species
k_1	Limiting retention factor for completely solvated analyte
k_2	Limiting retention factor for desolvated analyte
K	Solvation constant

8-REFERENCES

1 C. Horvath, W. Melanderm and I Molnar, *Anal. Chem* 49(1977)142-154.

2 J.H. Knox and G.R. Laird , *J. Chromatogr.* 122(1976)17-34.

3 J.C. Kraak, K.M. Jonker, and J.F.K Huber, , *J. Chromatogr.* 142(1977)671-688.

4 Tesera cecchi, Ion-pair chromarography and related techniques, CRC press, 2010.

5 J.H. Knox and R.A. Hartwick,. *J. Chromatogr.* 204 (1981)3–21.

6 J.H. Knox and G.R. Laird, *J. Chromatogr.* 122 (1976):17–34.

7 C. Horvath. Melander, I. Molnar, and P. Molnar, *Anal. Chem.* 49 (1977)2295–2305.

8 J.C. Kraak, K.M. Jonker, and J.F.K. Huber, *J. Chromatogr.* 142 (1977)671–688.

9 Yu. S. Xianren and W. Baeyens, *J. Chromatogr.* 456 (1988)267-275.

10 C.T. Hung and R.B. Taylor, *J. Chromatogr.* 202 (1980)333–345.

11 B.A. Bidlingmeyer, *J. Chromatogr.* Sci. 18 (1980)525–539.

12 J. Stählberg, *J Chromatogr* , 356(1986) 231-245

13 J. Stahlberg and Furängen, *„J Chromatogr.* , 24 (1987) 783-789.

14 F.F. Cantwell and S. Puon, , *Anal. Chem.* 51 (1979) 623-632.

15 S.Afrashtehfar and F.F. Cantwell, Anal. Chem. 54(19820) 2422-2427.

16 F.F. Cantwell, *J. Pharmaceutical& Biomed. Anal.* 2(2) (1984)153–164.

17 T.V. Popa, C.T. Mant, Y. Chen, and R.S. Hodges,*J. Chromatogr. A* 1043 (2004)113-122.

18 W.R. Melander and C Horvath, in M.T.W. Hearn (ed.), Ion Pair Chromatography: Theory and Biological and Pharmaceutical Applications, Vol. 31, Marcel Dekker, New York, pp. 27-275.

19 Debye, P.; Hückel, E. Zur Theorie der Elektrolyte. 1. Gefrierpunktserniedrigung und vewandte Erscheinungen. Z. Phys. 24(1923)185–206.

20 Friedman, H. L. Ionic Solution Theory; Wiley: New York, 1962.

21 Pitzer, K. S. ActiVity Coefficients in Electrolyte Solutions, 2nd ed.;CRC Press: Boca Raton, FL, 1992.

22 Brönsted, J.N. *J. Am. Chem. Soc.* 42(1920)761-786

23 Bucher, R et al.*Chem. Phys. Chem.* 4(2003)373-378.

24 Davies,C.W . Ion Association. Butterworth: London,1962.

25 Fuoss, R.M ; Hsia, K.L.Proc. Natl, Acad. Sci. USA. 57(1967)1500-1557.

26 Fernandez-Prini, R. And Justice, *J.C. Pure Appl. Chem,* 56(1984)541-547.

27 Takayanagi , T., Wada E., and Motomizu, S. *Analyst* 122(1997)1387-1392

28 Takayanagi , T., Wada E., and Motomizu, S. *Analyst* 130(1998)1200-1211

29 Motomizu, S and Takayanagi, T. *J. Chromatogr. A.* 853(1999)63-69.

30 Martell, A.E. And Motekaitis, R.J. VCH:New York, 1992.

31 Kaatze,U., Hushcha, T.O., and Eggers,*J. Solution Chem.* 29(2000)299-368.

32 Chen,T.,Hefter,G., and Buchner,R. *J. Phys. Chem.* 107(2003)4025-4031.

33 Buhner,R., Chen,T., and Hefter,G. *J. Phys.Chem.B.* 108(2004)2365-2375.

34 Hefter,G.T.*Pure Appl. Chem.* 78(2006)1571-1586.

35 J.H. Knox and R.A. Hartwick, *J. Chromatogr.* 204(1981)3-21.

36 R. LoBrutto, A. Jones, and Y. Kazakevich, *J. Chromatogr. A* 913(2001)189-196.

37 R.Lo Brutto and Y.K. Kazakevich, Adv Chromatogr, 44(2006)291-314.

38 R.Lo Brutto and Y.K. Kazakevich, Effect of chaotropic anions on analyte retention, in Proceedings, 22nd International Symposium on High Performance Liquid Phase Separations and Related Techniques, St. Louis , May 2-8,1998.

39 Rydall JR, Macdonald PM.. *Biochem* 31(1992)1092-1099.

40 Hatefi Y, Hanstein WG.. *Proc Natl Acad Sci USA* 62(1969)1129-1136.

41 Kunz W, Henle J, Ninham BW. Zur Lehre von der Wirkung der Salze. *Curr Opin Colloid Interface Sci* 9(2004)19-37.

42 Cecchi T, Passamonti P.. *J Chromatogr A* 1216(2009)1789-1797.

43 J. Li, *Anal. Chim. Acta* 369(1–2) (1998)21–37.

44 Collins, K. *J Biophys..* 77(1997)65-76.

45 Kazakevich YV, LoBrutto R, Vivilecchia R. *J Chromatogr A.* 1064 (2005)9-18.

46 F. Gritti and G Guiochon, *J Chromatogr. A* 1038 (2004)53-66.

47 F. Gritti and G Guiochon, J. Chromatogr. A 1033 (2004)43-55.

48 F. Gritti and G. Guiochon, *J Chromatogr. A* 1033(2004)57-69.

49 C. Horvath, W. Melander, I. Molnar, and P. Molnar, *Anal. Chem.* 49(1977)2295-2305.

50 A. Sokolowski,. *Chromatographia* 22(1986)168-173.

51 A. Sokolowski, *Chromatographia* 22 (1986)177-183.

52 I. Hagglund and J. Stählberg, *J. Chromatogr. A* 761(1997) 3-47

53 Hofmeister F. *Arch. exp. Pathol. Pharmakol.* 24(1888)247–260.

54 Collins KD, Washabaugh MW. *Q Rev Biophys.* 18(1985)323–422.

55 Zhang Y, Cremer PS. *Curr Opin Chem Biol.* 10(2006)658–663

56 Wilson EK. *Chemical & Engineering News.* 85(2007)47–49.

57 Cacace MG, Landau EM, Ramsden JJ. *Q Rev Biophys.* 30(1997)241–277.

58 Leberman R, Soper AK. *Nature.* 378(1995)364–366.

59 Collins KD. *Biophys J.* 72(1997)65–76.

60 Robinson RA, Stockes RH. *Butterworth Scientific Publications; London, 1959.* 1959

61 Breslow R, Guo T. *Proc Natl Acad Sci U S A.* 87(1990)167–169

62 Kunz W, Belloni L, Bernard O, Ninham BW. *J. Phys. Chem. B.* 108(2004)2398–2404.

63 Ohtaki H, Radnai T. *Chem Rev.* 93(1993)1157–1204.

64 Dillon SR, Dougherty RC. *J. Phys. Chem. A.* 106(2002)7647–7650

65 Nickolov ZS, Miller JD. *J Colloid Interface Sci.* 287(2005)572–580.

66 Yamaguchi T, Lindqvist O, Claeson T, Boyce JB. *Chemical Physics Letters.* 93(1982)528–532.

67 Yamaguchi T, Niihara M, Takamuku T, Wakita H, Kanno H. *Chemical Physics Letters.* 274(1997)485–490.

68 Cappa CD, Smith JD, Messer BM, Cohen RC, Saykally RJ. *J. Phys. Chem. B.* 110(2006)5301–5309.

69 Omta AW, Kropman MF, Woutersen S, Bakker HJ. *Science.* 301(2003)347–349.

70 Kropman MF, Bakker HJ. *Science.* 291(2001)2118–2120.

71 Batchelor JD, Olteanu A, Tripathy A, Pielak GJ. *J. Am. Chem. Soc.* 126(2004)1958–1961.

72 Naslund LA, Edwards DC, Wernet P, Bergmann U, Ogasawara H, Pettersson LGM, Myneni S, Nilsson A. *J. Phys. Chem. A.* 109(2005)5995–6002.

73 Bakker HJ, Kropman MF, Omta AW. *J. Physics: Condensed Matter.* 17(2005)S3215.

74 Smith JD, Saykally RJ, Geissler PL. *J. Am. Chem. Soc.* 129(2007)13847–13856.

75 Hribar B, Southall NT, Vlachy V, Dill KA. *J. Am Chem Soc.* 124(2002)12302–12311

76 Thomas AS, Elcock AH. *J. Am. Chem. Soc.* 129(2007)14887–14898.

77 Chen X, Yang T, Kataoka S, Cremer PS. *J. Am. Chem. Soc.* 129(2007)12272–12279

78 Frauenfelder H, McMahon BH, Fenimore PW. *Proc Natl Acad Sci U S A.* 100(2003)8615–8617.

79 Fenimore PW, Frauenfelder H, McMahon BH, Parak FG. *Proc Natl Acad Sci U S A.* 99(2002)16047–16051.

80 Samuni U, Dantsker D, Roche CJ, Friedman JM. *Gene.* 398(2007)234–248.

81 Frauenfelder H, Fenimore PW, Chen G, McMahon BH. *Proc Natl Acad Sci U S A.* 103(2006)15469–15472.

82 Samuni U, Roche CJ, Dantsker D, Friedman JM. *J Am Chem Soc.* 129(2007)12756–12764.

83 Henzler-Wildman KA, Lei M, Thai V, Kerns SJ, Karplus M, Kern D. *Nature.* 450(2007)913–916.

84 Roberts J, Diaz. Fortin D, Friedle J and Piper S, *Anal Chem.* 74(2002)4927-4932.

85 I.L. Kazakevich, PhD. Thesis, Seton Hall University, NJ, 2005.

86 I.L. Kazakevich, N. H. Snow, Adsorption behaviour of hexafluorophosphate on selected bonded phases, *J. Chromatogr. A* 119(2006)43-50.

87 A. Giaquinto, Z. Liu, A. Bach, Y.Kazakevich *Anal. Chem.* 80 (2008) 6358-6364.

88 Y.V.Kazakevich, R. LoBrutto, F. Chan, and T. Patel, *J. Chromatogr. A* 913(2001)75-87.

89 J. Stählberg, *„J Chromatogr A,* 855 (1999) 3-55

90 Bjerrum, N. Kgl. *Danske Videnskab Selskab*, 7(1926)1–48.

91 Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* 24(1888)247–260.

92 Kunz, W.; Henle, J.; Ninham, B. W. *Curr. Opin. Colloid Interf. Sci.* 9(2004)19–37.

93 H.S. Harned, B.B. Owen, The physical chemistry of electrolytic solutions, ReinholdPub. Corp., New York, 1958.

94 A.L. Horvath, Handbook of aqueous electrolyte solutions: physical properties,estimation, and correlation methods, Ellis Horwood, Halsted Press, Chichester NewYork, 1985.

95 A. Kopatsis, Thermodynamics of electrolyte solutions: the isoactive solution theory,1985.

96 S.A. Rice, M. Nagasawa, Polyelectrolyte solutions, a theoretical introduction, Academic Press, London, 1961.

97 V. Vlachy, B. Hribar-Lee, Y.V. Kalyuzhnyi, K.A. Dill, *Curr Opin Colloid Interface Sci* 9(2004) 128-136.

98 W. Kunz, P. Lo Nostro, B.W. Ninham, *Curr Opin Colloid Interface Sci* 9 (2004) 1-7.

99 M. Bostrom, D.R.M. Williams, B.W. Ninham, *Curr Opin Colloid Interface Sci* 9(2004) 48-56.

100 G. Ahn-Ercan, H. Krienke, W. Kunz, *Curr Opin Colloid Interface Sci* 9(2004)92-99.

101 V. Vlachy, B. Hribar-Lee, Y.V. Kalyuzhnyi, K.A. Dill, *Curr Opin Colloid Interface Sci* 9(2004) 128-133.

102 M.G. Cacace, E.M. Landau, J.J. Ramsden, *Q Rev Biophys* 30 (1997) 241-250

103 K.D. Collins, *Biophys Chem* 119 (2006) 271-299.

104 P. Westh, H. Kato, K. Nishikawa, Y. Koga, *J Phys Chem A* 110 (2006) 2072-2085.

105 R. Leberman, A.K. Soper, *Nature* 378 (1995) 364-374.

106 L.M. Pegram, M.T. Record Jr., *J Phys Chem B* 111 (2007) 5411-5420.

107 K.D. Collins, G.W. Neilson, J.E. Enderby, *Biophys Chem* 128 (2007) 95-103.

108K.D. Collins, *J. Biophys.* 72 (1997) 65-74.

109 S. Raugei, M.L. Klein, *J Chem Phys* 116 (2002) 196-209.

110 R.L. Mancera, *J Phys Chem B* 103 (1999) 3774-3785.

111 Y. Marcus, *Biophys Chem* 51 (1994) 111-120.

112 I. Rustamov , T. Farcas , F. Ahmed , F. Chan , R. LoBrutto , H.M. McNair ,Y.V. Kazakevich ,*J. ChromatogrA.* 613 (2001) 49-63.

113 R. D. Oparin, M. V. Fedotova and V. N. Trostin, *J. Structural Chem.* 43 (2002) 467-472.

114 Coym,J.W. and Dorsey, J.G. *J. Chromatrogr. A.* 1035(2004)23-29

9-Appendix

Liophilic ion		Salt concentration(mM)					
	0	1	10	20	30	40	50
CLO_4^-	0.79	1.52	2.24	2.68	2.95	3.15	3.30
SCN^-	0.79	1.26	2.02	2.50	2.77	2.97	3.10
CH_3COO^-	0.79	0.89	1.13	1.33	1.38	1.40	1.43
$HCOO^-$	0.79	1.10	1.27	1.34	1.36	1.38	1.40
Cl^-	0.79	0.87	1.15	1.23	1.26	1.29	1.30
$HC_6H_5O_7^{-2}$	0.79	0.95	1.19	1.25	1.26	1.28	1.30
SO_4^{-2}	0.79	0.99	1.20	1.25	1.25	1.24	1.25
$H_2PO_4^-$	0.79	0.83	0.94	1.03	1.09	1.12	1.15

Table 5A Retention time for PTMA-Cl as a function of added salt in water/acetonitrile mobile phase

Liophilic ion		Salt concentration(mM)					
	0	1	10	20	30	40	50
SCN^-	1.39	1.92	2.45	2.91	3.26	3.55	3.72
CLO_4^-	1.39	1.89	2.33	2.80	3.11	3.35	3.51
CH_3COO^-	1.39	1.92	2.22	2.24	2.25	2.27	2.29
$HCOO^-$	1.39	1.58	1.66	1.74	1.81	1.85	1.88
$HC_6H_5O_7^{-2}$	1.39	1.56	1.63	1.70	1.75	1.78	1.81
Cl^-	1.39	1.52	1.59	1.65	1.68	1.71	1.74
SO_4^{-2}	1.39	1.49	1.57	1.62	1.65	1.66	1.69
$H_2PO_4^-$	1.39	1.46	1.53	1.56	1.59	1.62	1.64

Table 6A. Retention time for PTMA-Cl as a function of added salt in water/methanol mobile phase

	Radius	Enthalpy aq	Gibbs en aq	B- coeff	Hydr num	Volume aq	Charge
	(pm)	(kJ mol^{-1})	(kJ mol^{-1})	(cm^3mol^{-1})		(cm^3mol^{-1})	
SCN^-	213	76.44	92.71	-0.103	1.7	41.2	1
CLO_4^-	240	-129.33	-8.52	-0.061	1.4	49.6	1
CH_3COO^-	232	-486.01	-369.31	0.246	2.2	46.2	1
Cl^-	181	-167.16	-131.2	-0.005	2	23.3	1
$HCOO^-$	204	-425.55	-351	0.052	2.1	31.6	1
$H_2PO_4^-$	200	-1296.29	-1130	0.34	1.8	34.6	1
SO_4^{-2}	230	-909.27	-744.5	0.206	3.1	25	2
HPO_4^{-2}	200	-1292.14	-1089	0.382	3.6	18.7	2

Table 7A. Thermodynamic properties for the ions studied.

Formate, phosphate and perchlorate data taken from Krestov(1991)

All others from Robinson et al(1981)

Aniline	pH 3.0 Salt concentration(mM)					
	1	**5**	**15**	**20**	**30**	**100**
CH_3COONH_4	1.05	1.16	1.17	1.14	1.14	1.08
$HCOONH_4$	0.91	1.09	1.17	1.20	1.23	1.39
$SCNNH_4$	1.17	1.28	1.66	1.85	2.02	2.55
K_2SO_4	1.07	1.19	1.31	1.27	1.28	1.31
KH_2PO_4	0.87	1.02	1.17	1.18	1.20	1.29
NH_4Cl	0.86	1.00	1.15	1.18	1.24	1.36
NaCl	0.88	1.05	1.22	1.19	1.25	1.37

Table 8A. Retention of aniline as a function of salt concentration in the mobile phase at pH 3.0

Pyridine	pH 3.0 Salt concentration(mM)					
	1	**5**	**15**	**20**	**30**	**100**
CH_3COONH_4	0.63	0.705	0.742	0.754	0.78	0.8
$HCOONH_4$	0.617	0.683	0.729	0.736	0.752	0.803
$SCNNH_4$	0.688	0.754	0.887	0.946	0.968	1.047
K_2SO_4	0.685	0.734	0.763	0.755	0.756	0.764
KH_2PO_4	0.61	0.671	0.724	0.732	0.74	0.762
NH_4Cl	0.606	0.659	0.719	0.733	0.749	0.781
NaCl	0.618	0.684	0.746	0.741	0.755	0.786

Table 9A. Retention of Pyridine as a function of salt concentration in the mobile phase at pH 3.0

PTMA-Cl pH 3.0

salt conc	0	1	5	15	20	30	50	100
CH_3COONH_4	0.38	0.70	0.74	1.06	1.11	1.16	1.17	1.19
$HCOONH_4$	0.38	0.61	0.81	1.22	1.33	1.44	1.48	1.54
$SCNNH_4$	0.38	1.27	2.50	3.46	3.68	4.14	4.40	4.55
K_2SO_4	0.38	0.37	0.62	0.67	0.69	0.70	0.72	0.74
KH_2PO_4	0.38	0.55	0.64	0.67	0.67	0.68	0.69	0.69
NaCl	0.38	0.43	0.71	1.03	1.10	1.21	1.37	1.51
NH_4Cl	0.38	0.45	0.56	0.90	1.00	1.10	1.19	1.34

Table 10A. Retention of PTMA-Cl as a function of salt concentration in the mobile phase at pH 3.0

PTMA-Cl

%Acetonitrile in mobile phase	NH4CL in mobile phase				
	0mM	**1mM**	**5mM**	**15mM**	**50mM**
5	1.68	2.50	3.06	3.53	4.04
10	1.60	1.83	2.22	2.46	2.71
15	1.63	1.65	1.89	2.08	2.30
20	1.60	1.56	1.75	1.89	2.02
30	1.52	1.45	1.58	1.68	1.77

Table 11A. Retention of PTMA-Cl as a function of salt concentration in the mobile phase

PTMA-Br

%Acetonitrile in mobile phase	NH4CL in mobile phase				
	0mM	**1mM**	**5mM**	**15mM**	**50mM**
5	1.51	2.32	3.12	3.58	4.08
10	1.53	1.78	2.25	2.48	2.73
15	1.56	1.62	1.91	2.11	2.31
20	1.50	1.52	1.75	1.88	2.01
30	1.44	1.41	1.56	1.67	1.77

Table 12A. Retention of PTMA-Br as a function of salt concentration in the mobile phase

PTMA-I

%Acetonitrile	NH4CL in mobile phase				
in mobile phase	**0mM**	**1mM**	**5mM**	**15mM**	**50mM**
5	1.44	2.27	3.15	3.62	4.11
10	1.45	1.75	2.31	2.52	2.75
15	1.45	1.58	1.92	2.12	2.32
20	1.40	1.49	1.74	1.90	2.03
30	1.37	1.38	1.56	1.67	1.78

Table 13A. Retention of PTMA-I as a function of salt concentration in the mobile phase

	Thickness of Adsorbed layer	
% organic	Acetonitrile	Methanol
0	0	0
0.01	0.893	0.394
0.05	3.36	1.572
0.1	5.707	2.489
0.2	9.747	3.542
0.3	12.978	4.054
0.4	15.184	4.294
0.5	16.273	4.366
0.6	16.577	4.361
0.7	16.598	4.352
0.8	16.746	4.438
0.9	17.394	4.668
0.95	18.048	4.871
0.99	18.945	5.16
1.00	19.293	5.329

Table 14A Thickness of Adsorbed layer

Retention time for $PTMA^+$ Column Temperature(°C)	% of Acetonitrile in mobile phase									
	2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
5°C	3.52	2.68	2.12	1.76	1.50	1.32	1.18	1.09	1.09	0.96
10°C	3.22	2.49	1.98	1.66	1.43	1.27	1.15	1.06	1.06	0.95
15°C	2.97	2.31	1.85	1.57	1.36	1.22	1.12	1.04	1.04	0.93
20°C	2.78	2.18	1.77	1.51	1.33	1.20	1.10	1.03	1.03	0.93
25°C	2.58	2.04	1.69	1.46	1.29	1.18	1.09	1.02	1.02	0.93
30°C	2.42	1.93	1.61	1.41	1.27	1.15	1.08	1.01	1.01	0.93
35°C	2.29	1.86	1.57	1.38	1.25	1.15	1.07	1.01	1.01	0.93
40°C	2.17	1.77	1.51	1.34	1.22	1.13	1.06	1.00	1.00	0.93
45°C	2.06	1.70	1.46	1.32	1.20	1.12	1.05	1.00	1.00	0.93
50°C	1.96	1.64	1.43	1.29	1.19	1.12	1.05	1.01	1.00	0.94

Table 15A. Retention time of PTMA+ as a function of temperature at different organic modifier concentrations with 20 mM of NH_4SCN.

Adsorption calculations for column characterization.

$$\begin{pmatrix} m_{ads} \\ p_{cal} \\ p_{ads} \\ p_{des} \end{pmatrix} :=$$

######## ######## ########
YMC PAC YMC PAC YMC PACI
A30D50 A30D50 A30D50

EXCEL component
experimental data
(paste exp. data in columns:
helium cal.- COL**A**;
adsorption - COL**B**;
desorption - COL**C**.
In the Output dialog box set address regions (e.g. A10:A302) etc.

$m_{ads} := m_{ads} \cdot g \quad m_{ads} = 0.0791\,g$

Experimental parameters

Manifold volume $V_{man} := 25.2746 \cdot mL$

Sample temperature $T_s := 77.2 \cdot K$

Manifold temperature $T_m := (25 + 273.2) \cdot K$

Nitrogen surface tension $\gamma := 8.72 \cdot \frac{dyne}{cm}$

Definitions

$nm := 10^{-9} \cdot m \qquad Å := 10^{-10} \cdot m$

$N_A := 6.022 \cdot 10^{23} \cdot \frac{1}{mole} \qquad mmole := 10^{-3} \cdot mole$

$R := 8.314472 \cdot \frac{joule}{mole \cdot K} \qquad \mu mole := 10^{-6} \cdot mole$

Liq. nitrogen molar volume $V_L := 34.68 \cdot \frac{mL}{mol}$

Sample vial volume calibration

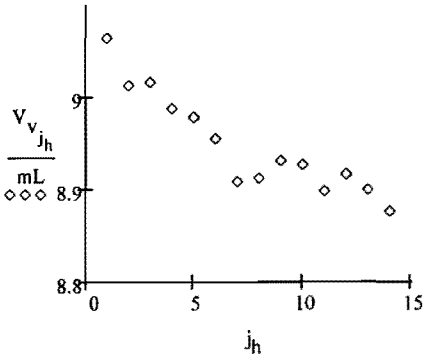
$p := p_{cal} \cdot torr \qquad n := \left| \frac{last(p) - 2}{3} \right|$ number of calibration points $n = 14$

$i := 0 .. \left| \frac{last(p) - 2}{3} \right| \qquad j_h := 1 .. n \qquad P_{oh_i} := p_{3 \cdot i} \qquad P_{eh_i} := p_{3 \cdot i + 1}$

$$V_{v_0} := \frac{T_s \cdot V_{man}}{T_m} \cdot \left(\frac{P_{oh_0}}{P_{eh_0}} - 1 \right) \qquad V_{v_{j_h}} := \frac{T_s \cdot V_{man}}{T_m} \cdot \frac{P_{eh_{j_h}} - P_{oh_{j_h}}}{P_{eh_{j_h - 1}} - P_{eh_{j_h}}}$$

$V_{av} := mean(V_v) \qquad V_{av} = 8.947\,mL$ Vial volume

$rsd\_V := \frac{stdev(V_v)}{V_{av}} \cdot 100 \quad rsd\_V = 0.578$ RSD%



Adsorption Isotherm

$p := p_{ads} \cdot torr \qquad n_a := \left| \frac{last(p) - 2}{3} \right| \qquad i := 0 .. n_a \qquad i2 := 1 .. n_a$

Split of a raw data series separate vectors: Po, Pe, Ps (adsorption data)

$po_i := p_{3 \cdot i} \qquad pe_i := p_{3 \cdot i+1} \qquad ps_i := p_{3 \cdot i+2}$

$sm_i := i \qquad pss := ksmooth(sm, ps, 11) \qquad pps_i := \frac{pe_i}{pss_i}$

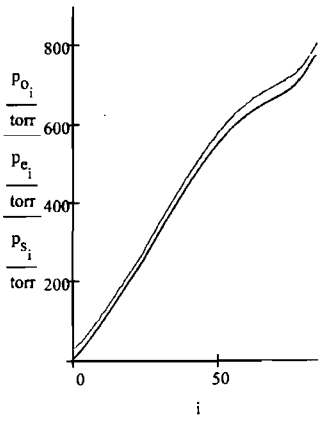
Smoothing of Ps data

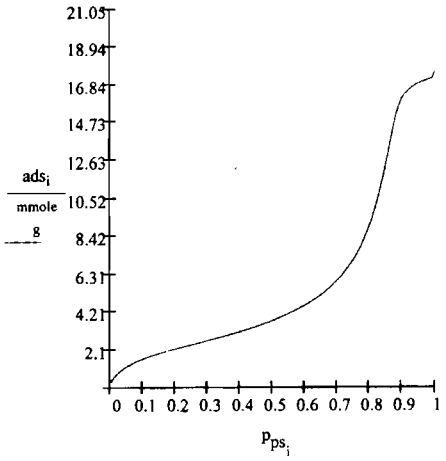
$$a_{ads_0} := \frac{po_0 \cdot V_{man}}{T_m} - \frac{pe_0 \cdot V_{man}}{T_m} - \frac{pe_0 \cdot V_{av}}{T_s}$$

$$a_{ads_{i2}} := \frac{V_{man}}{T_m} \cdot \left(po_{i2} - pe_{i2} \right) + \frac{V_{av}}{T_s} \cdot \left(pe_{i2-1} - pe_{i2} \right)$$

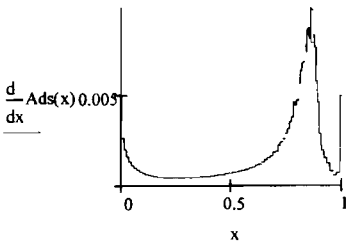
$$ads_i := \frac{\sum_{j2=0}^{i} a_{ads_{j2}}}{R \cdot m_{ads}}$$

$a_{max} := \max(ads) \qquad a_{max} = 17.539 \frac{mmole}{g}$





$Ads(x) := linterp(pps, ads, x)$



$V_{pore} := \frac{34.7}{1000} \cdot 16.847 \qquad V_p := V_{pore} \cdot \frac{mL}{g} \qquad V_p = 0.585 \frac{mL}{g}$

$\sqrt{\blacksquare}$

$$n_b(p_{ps}, n_a, mi, ma) := \begin{| i \leftarrow 0 \\ \text{for } j \in 0..n_a \\ \quad i \leftarrow i + 1 \text{ if } mi < p_{ps_j} < ma \\ i \end{|$$

$$n_s(p_{ps}, t) := \begin{| i \leftarrow 0 \\ \text{while } p_{ps_i} < t \\ \quad i \leftarrow i + 1 \end{|$$

BET calculations Select BET region (str - start; en - end)

str := .1 $n_s := n_s(p_{ps}, str)$ $n_s = 9$ en := .2 $n_b := n_b(p_{ps}, n_a, str, en)$ $n_b = 7$ $i_b := 0..n_b - 1$

$PP_{b_{i_b}} := p_{ps_{n_s+i_b}}$ $ads_{b_{i_b}} := ads_{n_s+i_b}$ $Bet_{i_b} := \dfrac{PP_{b_{i_b}}}{ads_{b_{i_b}} \cdot \left(1 - PP_{b_{i_b}}\right)}$

$slp := slope(PP_b, Bet)$ $icpt := intercept(PP_b, Bet)$ $C := \dfrac{slp}{icpt} + 1$ $N_{max} := \dfrac{1}{slp + icpt}$

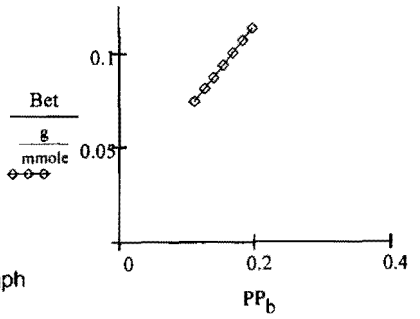
$\omega := 21 \cdot Å^2$ $S := \omega \cdot N_A \cdot N_{max}$

C = 20.609 Constant C of BET equation

$S = 260.429 \dfrac{m^2}{g}$ **surface area [m^2/g]**

$$ad_i := \frac{N_{max} \cdot C \cdot p_{ps_i}}{\left(1 - p_{ps_i}\right) \cdot \left[1 + (C - 1) \cdot p_{ps_i}\right]}$$

BET graph



$p := p_{des} \cdot torr$ **Pore volume calculations**

$n_p := \left| \frac{last(p) - 2}{3} \right|$ $i3 := 0 .. n_p$ $P_{po_{i3}} := p_{3 \cdot i3}$ $P_{pe_{i3}} := p_{3 \cdot i3+1}$ $P_{ps_{i3}} := p_{3 \cdot i3+2}$

$st_{i3} := i3$ $P_{ds} := ksmooth(st, P_{ps}, 10)$ Split and smooth of desorption raw data $P_{start} := pe_{n_a}$

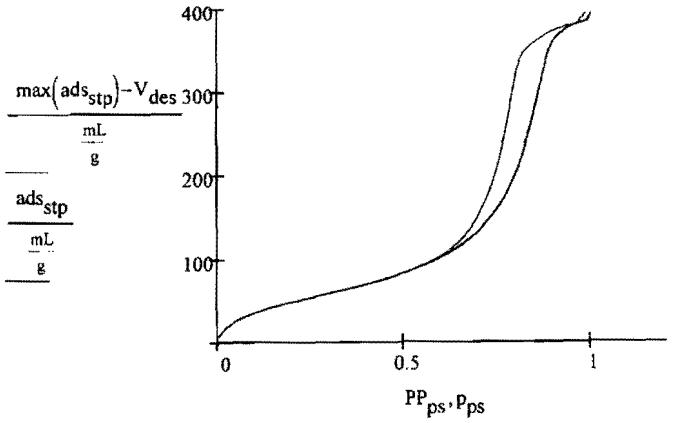
$PP_{ps_{i3}} := \frac{P_{pe_{i3}}}{P_{ds_{i3}}}$ $k := 1 .. n_p$ $PP_{ps} := PP_{ps}$ $n_p = 39$

$$v_{des_0} := \left[P_{pe_0} \cdot \left(\frac{V_{man}}{T_m} + \frac{V_{av}}{T_s} \right) - P_{po_0} \cdot \frac{V_{man}}{T_m} - P_{start} \cdot \frac{V_{av}}{T_s} \right] \cdot \frac{273.2 \cdot K}{760 \cdot torr \cdot m_{ads}}$$

$$v_{des_k} := \left[P_{pe_k} \cdot \left(\frac{V_{man}}{T_m} + \frac{V_{av}}{T_s} \right) - P_{po_k} \cdot \frac{V_{man}}{T_m} - P_{pe_{k-1}} \cdot \frac{V_{av}}{T_s} \right] \cdot \frac{273.2 \cdot K}{760 \cdot torr \cdot m_{ads}} \quad V_{des_k} := \left(\sum_{j=0}^{k} v_{des_j} \right)$$

$ads_{stp} := ads \cdot 273.2 \cdot K \cdot \frac{R}{1 \cdot atm}$

Full isotherm
adsorption and desorption branches
are shown in N2 volume at STP cond.



Evaluation of the adsorbtion thickness

$$opt := much(p_{ps}, 0.6) \qquad j_o := 0 .. opt - n_s \qquad vg := \begin{pmatrix} 3 \\ 2 \end{pmatrix}$$

$$ads_{u_{j_o}} := \frac{ads_{n_s + j_o}}{N_{max}} \qquad pp_{u_{j_o}} := p_{ps_{n_s + j_o}}$$

$$P := genfit(pp_u, ads_u, vg, F) \qquad P = \begin{pmatrix} 1.525 \\ 1.712 \end{pmatrix} \qquad ad_u(r) := F(r, P)_0$$

$$F(z,u) := \begin{bmatrix} \exp\left(\frac{\ln\left(\frac{u_1}{\ln\left(\frac{1}{z}\right)}\right)}{u_0}\right) \\ \frac{-\ln\left(\frac{u_1}{\ln\left(\frac{1}{z}\right)}\right)}{(u_0)^2} \cdot \exp\left(\frac{\ln\left(\frac{u_1}{\ln\left(\frac{1}{z}\right)}\right)}{u_0}\right) \\ \frac{1}{u_1 \cdot u_0} \cdot \exp\left(\frac{\ln\left(\frac{u_1}{\ln\left(\frac{1}{z}\right)}\right)}{u_0}\right) \end{bmatrix}$$

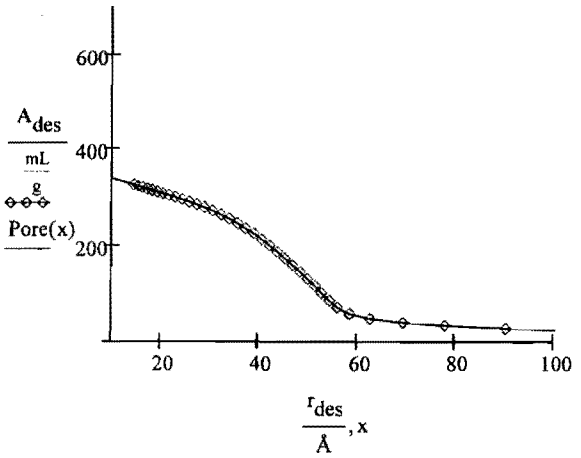
Nitrogen adsorbed layer thickness $\tau := 3.54 \cdot Å$

Pore size distribution calculations

$$A_{des} := reverse(V_{des}) \qquad \tau_{des_{i3}} := ad_u\left(PP_{ps_{i3}}\right) \cdot \tau \qquad R_{ds} := -\frac{\frac{2 \cdot \gamma \cdot V_L}{R \cdot T_s}}{\ln(PP_{ps})} \qquad R_{des_{i3}} := \tau_{des_{i3}} + R_{ds_{i3}}$$

$$r_{des} := reverse(R_{des}) \qquad Pore(x) := linterp\left(\frac{r_{des}}{Å}, \frac{A_{des}}{\frac{mL}{g}}, x\right)$$

Distribution (integral form)

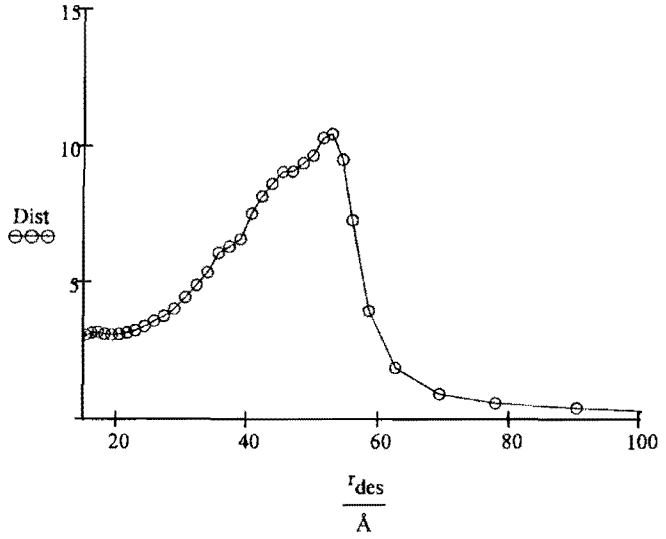


$$dis(x) := \frac{d}{dx} Pore(x)$$

$$Dist_{i3} := -dis\left(\frac{r_{des_{i3}}}{Å}\right)$$

$$R_{max} := r_{des_{much(Dist,\,max(Dist))+1}}$$

$$R_{max} = 52.72\,Å$$



Cylindrical pore correlation check

$$d_{Si} := 2.2 \cdot \frac{g}{mL} \qquad S = 260.429\,\frac{m^2}{g} \qquad V_p = 0.589\,\frac{mL}{g}$$

Radius from desorbtion $R_{max} = 52.72\,Å$

Radius from surface $2 \cdot \frac{V_p}{S} = 4.52\,nm$

$$\frac{R_{max} - 2 \cdot \frac{V_p}{S}}{R_{max}} \cdot 100 = 14.271$$

Geometry from hexagonal structure

$$D := \sqrt{\left(\frac{1}{d_{Si} \cdot V_p} + 1\right) \cdot \frac{2 \cdot \pi \cdot R_{max}^{\ 2}}{\sqrt{3}}} \qquad D = 13.368\,\text{nm}$$

Wall thickness

$$D - 2 \cdot R_{max} = 2.824\,\text{nm}$$

Pore volume calculation

$$d_{ads}(x) := \frac{d}{dx}Ads(x) \qquad i4 := 0..20 \qquad pr_{i4} := \frac{.25}{20}\cdot i4 + .75 \qquad da_{i4} := d_{ads}\left(pr_{i4}\right)$$

$$z := \frac{\min(da)}{\frac{mmole}{g}} \qquad z = 5.735 \qquad kl := lookup\left(z, \frac{da}{\frac{mmole}{g}}, pr\right) \qquad kl = \begin{pmatrix} 0.963 \\ 0.975 \end{pmatrix}$$

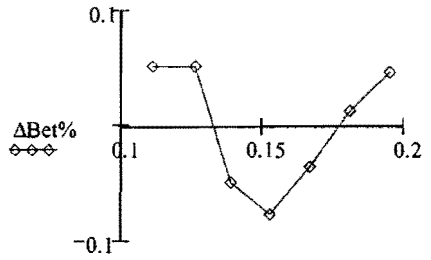
$$Ads(kl) = \begin{pmatrix} 16.97 \\ 17.042 \end{pmatrix}\frac{mmole}{g} \qquad V_{pv} := V_L \cdot Ads(kl) \qquad V_p = \begin{pmatrix} 0.589 \\ 0.591 \end{pmatrix}\frac{mL}{g} \qquad V_{pv} := V_{p_0}$$

find number for vector element < x

$$\text{much}(ads, x) := \begin{| i \leftarrow 0 \\ \text{while } ads_i < x \\ \quad i \leftarrow i + 1 \\ i - 1 |$$

1

$$\Delta Bet\%_{i_b} := \left[1 - \left(\frac{slp \cdot PP_{b_{i_b}} + icpt}{Bet_{i_b}}\right)\right] \cdot 100 \qquad N_{max} = 2.059 \frac{mmole}{g}$$



PP_b

t graph (relative to the standard isotherm from Gregg and Sing

$$\begin{pmatrix} pp_s \\ t_{val} \end{pmatrix} :=$$

1.00E-03	1.389
5.00E-03	1.875
0.01	2.152
0.02	2.673
0.03	2.951
0.04	3.124
0.05	3.229
0.06	3.263
0.07	3.367
0.08	3.472

$n_m := 5.381$ $\quad t(x) := \text{linterp}\left(\frac{t_{val}}{n_m}, pp_s, x\right)$

$Tf(x) := Ads(t(x))$